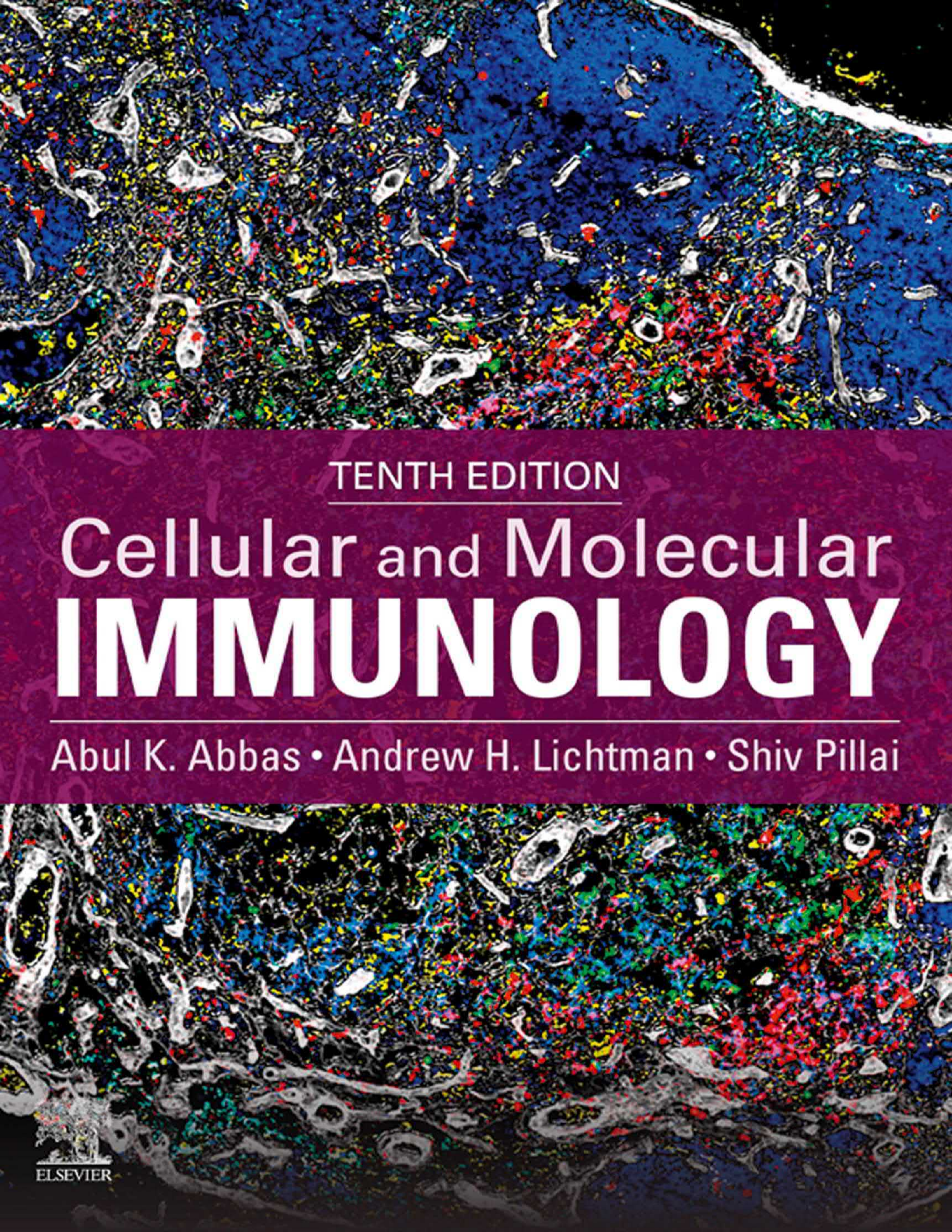

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Abul K. Abbas • Andrew H. Lichtman • Shiv Pillai

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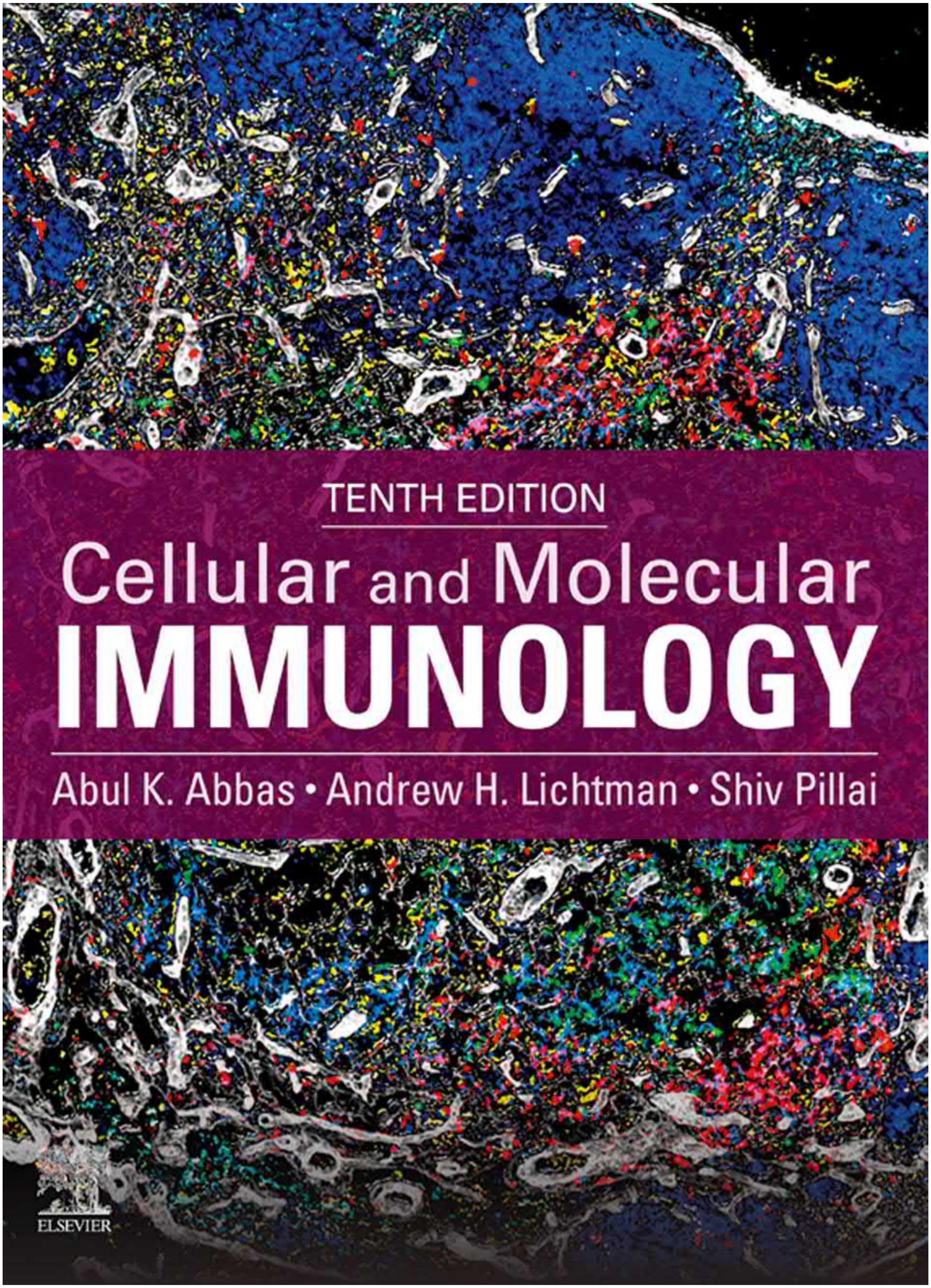

TENTH EDITION

Cellular and Molecular IMMUNOLOGY

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Cellular and Molecular Immunology

TENTH EDITION

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Dedication

To Our Students, Our Colleagues, and Our Families

Preface

This tenth edition of *Cellular and Molecular Immunology* includes substantial revisions, reflecting recent scientific advances and clinical applications of the science, while at the same time we have maintained the clear and readable style that has been typical of previous editions. Our presentation of new information focuses primarily on the important concepts and has not significantly increased the length of the book. We have also rewritten many sections for increased clarity, accuracy, and completeness.

The field of immunology has moved beyond establishing fundamental principles of the mechanisms of immune responses to applying these principles to understand and develop therapies for human disease. The revolution in immunological therapies over the last 20 years has been extraordinary. It is especially satisfying for immunologists that some of the most innovative and effective immunotherapies have been developed because the basic science has matured and the complex mechanisms of immune activation and regulation have been elucidated in increasing detail. Nowhere has the link between basic science and clinical medicine been demonstrated more forcefully than in the development of new cancer immunotherapies. Throughout the book, we have paid special attention to the clinical relevance of immunology and the scientific underpinnings of human disease and therapies. As we write this preface, the SARS CoV-2 pandemic has spread throughout the entire world. The science of immunology is of critical importance to the way we confront this challenge, and immunologists are front and center in learning how the virus causes disease, how we can treat it, and how we can develop effective vaccines.

In addition to these translational aspects of immunology, we have also updated basic concepts wherever there has been significant new understanding. Some examples of these fundamental advances include a better understanding of tissue resident macrophages and memory T cell subsets, mechanisms by which inflammasomes and nucleic acid sensors stimulate innate immune responses, the sequences of events in T cell–dependent antibody responses, and mechanisms by which tumor evasion of immune attack can be overcome

As in previous editions, each chapter is written so that it can be read and understood on its own, without referring to other chapters. To do this, it is often necessary to repeat some basic concepts and general principles that are covered in other chapters. We feel such repetition is valuable because it enables the reader to consolidate learning and understand the content of each chapter independently of the others. We also feel this is helpful for faculty teaching from the book because they can consider each chapter as the topic of one or two lectures.

We have also continued to improve our illustration program. Many illustrations have been revised to provide more visual depth and clarity. New figures have been added, and previously used figures have been reviewed and often changed for accuracy. We have kept design features such as the use of bold italic text to highlight "take-home messages" to make the book easy to read. We have tried to make the nomenclature more consistent by using the conventions for human genes and proteins wherever possible. The lists of suggested readings continue to emphasize recent review articles that provide in-depth coverage of particular topics for the interested reader. We have divided the lists into sections based on themes to help readers find the most useful articles for their needs. In this edition, we have added some classic primary research publications and links to the lectures of Nobel laureates in immunology. This edition also includes a listing of the online resources available to instructors and students (pages ix-x).

Individuals who have helped us with specific topics are (in alphabetical order) Drs. Bruce Bochner, Michael Carroll, Jason Cyster, Gaurav Gaiha, Michael Gerner, Florent Ginhoux, Amy Klion, Ari Molofsky, Robert Ohgami, and Andrea Radtke; all were generous with advice and images. Our illustrator, David Baker of DNA Illustrations, remains a full partner in the book and provides invaluable suggestions for clarity and accuracy. Several members of the Elsevier staff played critical roles. Our editor, Jeremy Bowes, has been a source of support and encouragement. Our managing editor, Rebecca Gruliow, shepherded the book through its preparation and into production. Ryan Cook was responsible for managing the design, and Clay Broeker was invaluable throughout the production stage. We also owe a debt of gratitude to our families for their unflagging support and their tolerance of our absences. Finally, our students were the original inspiration for the first edition of this book, and we remain continually grateful to them because from them we learn how to think about the science of immunology and how to communicate knowledge in the clearest and most meaningful way.

Abul K. Abbas

Andrew H. Lichtman

Shiv Pillai

Online Resources for Instructors and Students

Resources for Instructors

The following resources for instructors are available for use via Elsevier's Evolve website. Please contact your local sales representative for more information or go directly to the Evolve website to request access: https://evolve.elsevier.com . Note: It may take 1 to 3 days for account access setup and verification.

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All figures from *Cellular and Molecular Immunology*, edition 10, are available as an image collection in three formats (PowerPoint, JPEG, and PDF), with labels on and off. Figures may be downloaded individually or by chapter.

Test Bank

Instructors can access and download 251 UMSLE-style multiple-choice and matching questions from the test bank for use in classroom presentations and testing.

Resources for Students

The following resources are available online to students with the purchase of *Cellular and Molecular Immunology*, edition 10, on StudentConsult.com .

Textbook Online

The complete textbook is available online at *StudentConsult.com* . The online version is fully searchable and provides all figures from the print book, with enhanced functionality for many, including clickable enlargements and slideshow views of multiple-part images.

Glossary

The complete book glossary is available online at *StudentConsult.com* , with searchable terms linked to their discussion in the text. Readers may click on boldface highlighted key terms in the text to view pop-up definitions from the Glossary as they read the chapters online.

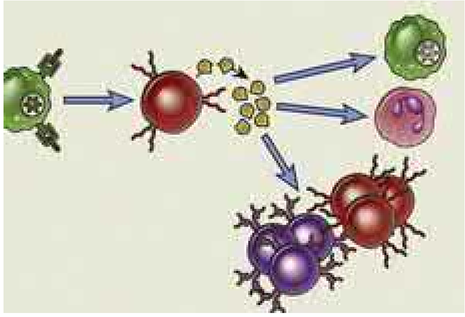
Clinical Cases

Five clinical cases are available online and linked via icons from the corresponding textbook discussion, indicated by the icon 🩺 in the margin. These clinical cases cover various diseases involving the immune system and are meant to show how the basic science of immunology contributes to our understanding of human diseases. Each case illustrates typical ways in which a disease manifests, what tests are used in diagnosis, and common modes of treatment. Each case poses questions and provides answers with explanations to increase understanding.

Self-Assessment Questions

Students can test and score themselves with 142 interactive multiple-choice questions available on *StudentConsult.com* .

Chapter 1: Properties and Overview of Immune Responses



Innate and Adaptive Immunity,

Innate Immunity,

Adaptive Immunity,
    Cardinal Features of Adaptive Immune Responses,
    Overview of Humoral and Cell-Mediated Immunity,
    Initiation and Development of Adaptive Immune Responses,
    Humoral Immunity,
    Cell-Mediated Immunity,

Summary,

The term **immunity** is derived from the Latin word *immunitas,* which referred to the protection from legal prosecution offered to Roman senators during their tenures in office. Historically, immunity meant protection from disease and, more specifically, infectious disease. The cells and molecules responsible for immunity constitute the **immune system,** and their collective and coordinated response to the introduction of foreign substances is called the **immune response.**

The physiologic function of the immune system is defense against infectious microbes; however, even noninfectious foreign substances and products of our own damaged and malignant (tumor) cells can elicit immune responses. Furthermore, mechanisms that normally protect individuals from infection and eliminate foreign substances also are capable of causing tissue injury and disease in some situations. In some situations, even self molecules can elicit immune responses (so-called autoimmune responses). Therefore, a more inclusive definition of the immune response is a reaction to microbes and to molecules that are recognized as foreign or abnormal, regardless of the physiologic or pathologic consequence of such a reaction. Immunology is the study of immune responses in this broader sense and of the cellular and molecular events that occur after an organism encounters microbes and other foreign macromolecules.

Historians often credit Thucydides, in the fifth century BC in Athens, as having first mentioned immunity to an infection that he called “plague” (but that was probably not the bubonic plague we recognize today). The concept of protective immunity may have existed long before, as suggested by the ancient Chinese custom of making children resistant to smallpox by having them inhale powders made from the skin lesions of patients recovering from the disease. Immunology, in its modern form, is an experimental science in which explanations of immunologic phenomena are based on experimental observations and the conclusions drawn from them. The development of immunology as an experimental discipline has depended on our ability to manipulate the function of the immune system under controlled conditions.

Historically, the first clear example of this manipulation, and one that remains among the most dramatic ever recorded, was Edward Jenner's successful vaccination against smallpox. Jenner, an English physician, was aware of an observation in rural England that milkmaids who had recovered from cowpox did not contract the more serious smallpox. On the basis of this observation, he injected the material from a cowpox pustule into the arm of an 8-year-old boy. When this boy was later intentionally inoculated with smallpox, the disease did not develop. Jenner's landmark treatise on **vaccination** (Latin *vaccinus,* of or from cows) was published in 1798. The principles of infectious diseases and vaccination were firmly established by the work of Louis Pasteur and Robert Koch a hundred years later. These advances led to the widespread acceptance of the method for inducing immunity, and vaccination remains the most effective strategy for preventing infections (Table 1.1). An eloquent testament to the importance of immunology was the announcement by the World Health Organization in 1980 that smallpox was the first disease that had been eradicated worldwide by a program of vaccination. The significance of the immune system has been dramatically and tragically highlighted by the AIDS (acquired immunodeficiency syndrome) epidemic, caused by HIV (human immunodeficiency virus), that started in the 1980s, and the COVID-19 pandemic caused by the coronavirus SARS-CoV-2, that started in 2019. Both have caused severe morbidity and many deaths, and have had a devastating impact on society. The development of effective vaccines for both diseases is a high priority.

TABLE 1.1

Disease	Maximum Number of Cases (Year)	Number of Cases in 2018	Percentage Change
Diphtheria	206,939 (1921)	1	−99.99
Measles	894,134 (1941)	375	−99.95
Mumps	152,209 (1968)	2,515	−95.82
Pertussis	265,269 (1934)	15, 609	−94.11
Polio (paralytic)	21,269 (1952)	0	−100.0
Rubella	57,686 (1969)	4	−99.99
Tetanus	1,560 (1923)	23	−98.52
***Haemophilus influenzae* type B**	~20,000 (1984)	38	−99.83
Hepatitis B	26,611 (1985)	3,322	−87.51

This table illustrates the striking decrease in the incidence of selected infectious diseases in the United States for which effective vaccines have been developed.

Data from Orenstein WA, Hinman AR, Bart KJ, Hadler SC. Immunization. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practices of Infectious Diseases.* 4th ed. New York, NY: Churchill Livingstone; 1995; and *Nationally Notifiable Infectious Diseases and Conditions, United States: 2018 Annual Tables*.

Since the 1960s, there has been a remarkable transformation in our understanding of the immune system and its functions. Advances in cell culture techniques (including monoclonal antibody production), immunochemistry, recombinant DNA methodology, next-generation DNA sequencing, x-ray crystallography, and creation of genetically altered animals (especially transgenic and knockout mice) have changed immunology from a largely descriptive science into one in which diverse immune phenomena can be explained in structural and biochemical terms. Some of the most important advances in immunology have come since the 1990s, with the development of therapies targeting different components of the immune system that are based on fundamental science and are dramatically altering the progression of human inflammatory diseases and cancers.

In this chapter, we outline the general features of immune responses and introduce the concepts that form the cornerstones of modern immunology and that recur throughout this book.

Innate and Adaptive Immunity

Defense against microbes is mediated by sequential and coordinated responses that are called innate and adaptive immunity (Fig. 1.1 and Table 1.2)**. Innate immunity** (also called natural immunity or native immunity) is essential for defending against microbes in the first few hours or days after infection, before adaptive immune responses have developed. Innate immunity is mediated by mechanisms that are in place even before an infection occurs (hence innate) and are capable of reacting rapidly to invading microbes.

In contrast to innate immunity, there are other immune responses that are stimulated by exposure to infectious agents and increase in magnitude and defensive capabilities with each successive exposure to a particular microbe. Because this form of immunity develops as a response to infection and thus adapts to the infection, it is called **adaptive immunity** (also called specific immunity or acquired immunity). The adaptive immune system recognizes and reacts to a large number of microbial and nonmicrobial substances, called **antigens.** Although many pathogens have evolved to resist the innate immune response, the stronger and more specialized adaptive immune responses are capable of eradicating many of these infections. There are also numerous connections between innate and adaptive immune responses. The innate immune response to microbes provides early danger signals that stimulate adaptive immune responses. Conversely, adaptive immune responses often work by enhancing the protective mechanisms of innate immunity, making them more capable of effectively combating microbes.

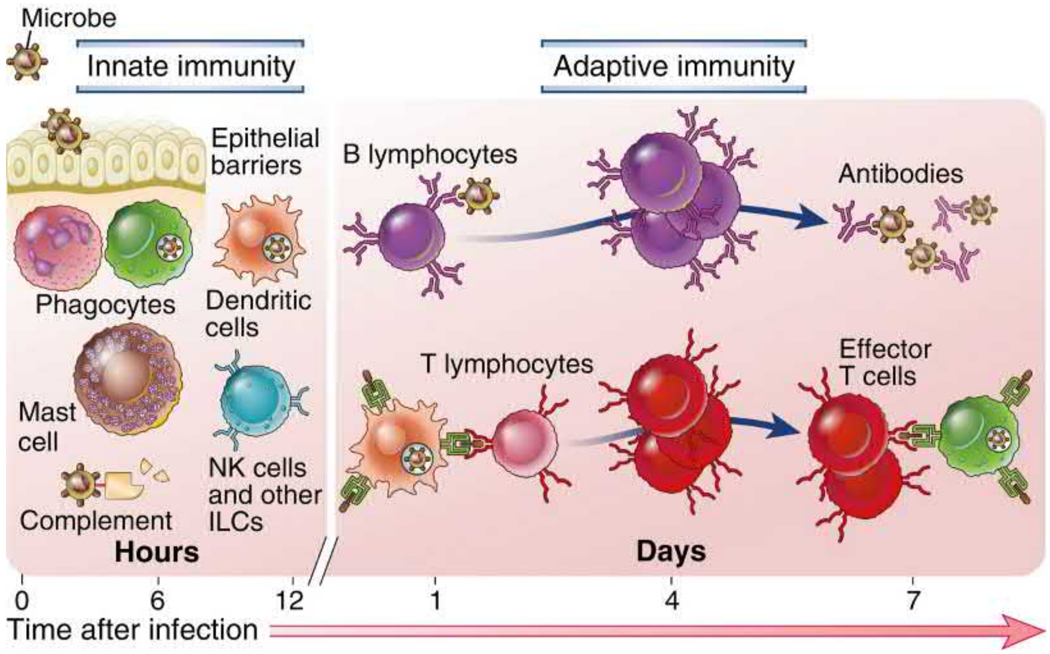

Microbe
Innate immunity
Adaptive immunity
Epithelial barriers
Phagocytes
Dendritic cells
Mast cell
NK cells and other ILCs
Complement
Hours
B lymphocytes
Antibodies
T lymphocytes
Effector T cells
Days
0
6
12
1
4
7
Time after infection

FIGURE 1.1 Innate and adaptive immunity. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and require the activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections. Only selected cell types are shown. *ILCs,* Innate lymphoid cells; *NK,* natural killer.

TABLE 1.2

	Innate	Adaptive
Characteristics		
Specificity	For molecules shared by groups of related microbes and molecules produced by damaged host cells	For many different microbial and nonmicrobial antigens
Diversity	Low; recognition molecules encoded by inherited (germline) genes	Very high; many antigen receptors are generated by somatic recombination of gene segments in lymphocytes
Memory	Limited	Yes
Nonreactivity to self	Yes	Yes
Components		
Cellular and chemical barriers	Skin, mucosal epithelia; antimicrobial molecules	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Secreted proteins	Complement, various lectins	Antibodies
Cells	Phagocytes (macrophages, neutrophils), dendritic cells, natural killer cells, mast cells, innate lymphoid cells	Lymphocytes

Many of the components of innate immunity also serve important functions in adaptive immune responses, as we will discuss in later chapters.

Every individual's immune system is able to recognize, respond to, and eliminate many foreign (nonself) antigens but does not usually react against that individual's own (self) antigens and tissues. Different mechanisms are used by the innate and adaptive immune systems to prevent reactions against healthy host cells.

Mechanisms for defending the host against microbes are present in all multicellular organisms. The phylogenetically oldest mechanisms of host defense are those of innate immunity, which are present even in plants and insects. Approximately 500 million years ago, jawless fish, such as lampreys and hagfish, developed an immune system containing lymphocyte-like cells that may function like lymphocytes in more advanced species and even respond to immunization. The antigen receptors on these cells are proteins with limited variability that are capable of recognizing many antigens but are distinct from the highly variable antibodies and T cell receptors that appeared later in evolution. The more specialized defense mechanisms that constitute adaptive immunity are found only in vertebrates. Most of the components of the adaptive immune system, including lymphocytes with diverse antigen receptors, antibodies, and specialized lymphoid tissues, evolved coordinately within a short time in jawed vertebrates (e.g., sharks) approximately 360 million years ago.

Innate Immunity

The innate immune system responds almost immediately to microbes and injured cells, and repeated exposures induce virtually identical innate immune responses. The receptors of innate immunity are specific for structures that are common to groups of related microbes and do not distinguish fine differences among microbes. The principal components of innate immunity are (1) physical and chemical barriers, such as epithelia and antimicrobial chemicals produced at epithelial surfaces; (2) phagocytic cells (neutrophils, macrophages), dendritic cells (DCs), mast cells, natural killer (NK cells), and other innate lymphoid cells; and (3) blood proteins, including components of the complement system and other mediators of inflammation. Many innate immune cells, such as DCs, some macrophages, and mast cells, are tissue resident, and they function as sentinels to keep watch for microbes that may invade the tissues. The innate immune response combats microbes by two main strategies—by recruiting phagocytes and other leukocytes that destroy the microbes, in the process called **inflammation;** and by blocking viral replication or killing virus-infected cells by mechanisms distinct from inflammatory reactions. We will discuss the features, mechanisms, and components of innate immunity in Chapter 4.

Adaptive Immunity

The adaptive immune response is mediated by cells called lymphocytes and their products. Lymphocytes express highly diverse receptors that are capable of recognizing a vast number of antigens. There are two major populations of lymphocytes, called **B lymphocytes** and **T lymphocytes,** which mediate different types of adaptive immune responses. We will first summarize the important properties of the adaptive immune system and then describe the different types of adaptive immune responses.

Cardinal Features of Adaptive Immune Responses

The fundamental properties of the adaptive immune system reflect the properties of the lymphocytes that mediate these responses.

- ***Specificity and diversity.*** Immune responses are specific for distinct antigens and often for different portions of a single complex protein, polysaccharide, or other macromolecule (Fig. 1.2). The parts of complex antigens that are specifically recognized by lymphocytes are called **determinants** or **epitopes.** This fine specificity exists because individual lymphocytes express membrane receptors that can distinguish subtle structural differences between distinct epitopes. Clones of lymphocytes with different specificities are present in unimmunized individuals and are able to recognize and respond to foreign antigens (Fig. 1.3). This fundamental concept is called **clonal selection.** It was clearly enunciated by Macfarlane Burnet in 1957 as a hypothesis to explain how the immune system could respond to a large number and variety of antigens. According to this hypothesis, which is now a proven feature of adaptive immunity, antigen-specific clones of lymphocytes develop before and independent of exposure to antigen. An introduced antigen binds to (selects) the cells of the preexisting antigen-specific clone and activates them, leading to an immune response specific for that antigen. The total number of antigenic specificities of the lymphocytes in an individual, called the lymphocyte repertoire, is extremely large. It is estimated that the immune system of an individual can discriminate 10^7 to 10^9 distinct antigenic determinants. This ability of the lymphocyte repertoire to recognize a very large number of antigens, called **diversity,** is the result of variability in the structures of the antigen-binding sites of lymphocyte receptors for antigens. In other words, there are many different clones of lymphocytes and each clone has a unique antigen receptor and therefore a singular antigen specificity, contributing to a total repertoire that is extremely diverse. The expression of different antigen receptors in different clones of T and B cells is the reason why these receptors are said to be clonally distributed. The molecular mechanisms that generate such diverse antigen receptors are discussed in Chapter 8. Diversity is essential if the immune system is to defend individuals against the many potential pathogens in the environment.

- ***Memory.*** Exposure of the immune system to a foreign antigen enhances its ability to respond again to that antigen. Responses to second and subsequent exposures to the same antigen, called secondary immune responses, are usually more rapid, greater in magnitude, and often qualitatively different from the first, or primary, immune response to that antigen (see Fig. 1.2). Immunologic memory occurs because each exposure to an antigen generates long-lived memory cells specific for the antigen. There are two reasons why secondary responses are typically stronger than primary immune responses—memory cells accumulate and become more numerous than the naive lymphocytes specific for the antigen that exist at the time of initial antigen exposure, and memory cells react more rapidly and vigorously to antigen challenge than do naive lymphocytes. Memory enables the immune system to mount heightened responses to persistent or recurring exposure to the same antigen and thus to combat infections by microbes that are prevalent in the environment and are encountered repeatedly.

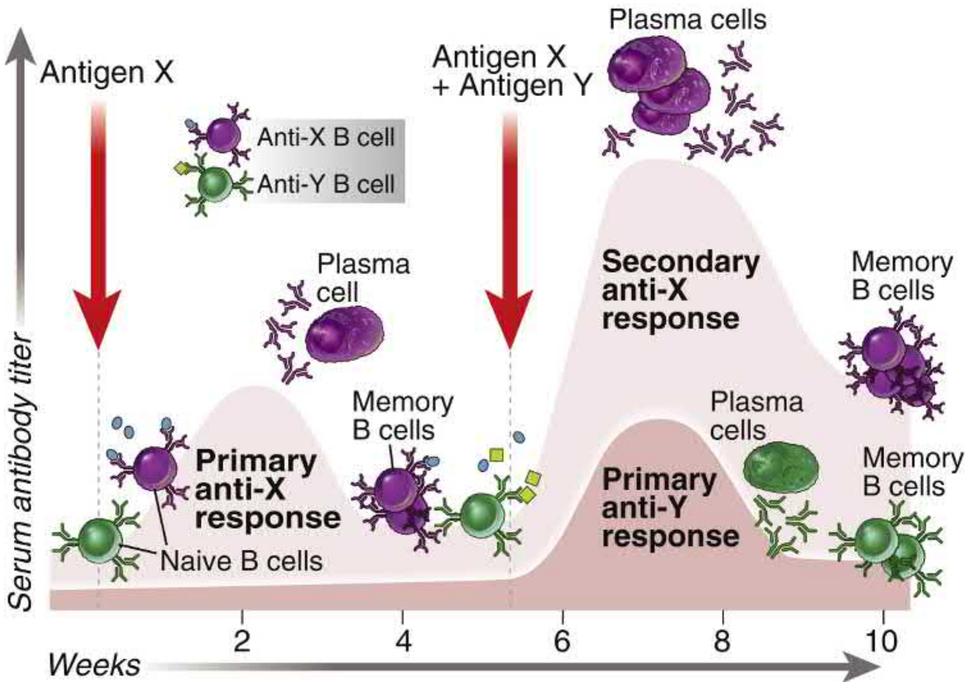

Antigen X
Anti-X B cell
Anti-Y B cell
Plasma cell
Primary anti-X response
Naive B cells
Memory B cells
Antigen X + Antigen Y
Plasma cells
Secondary anti-X response
Memory B cells
Plasma cells
Primary anti-Y response
Memory B cells
Serum antibody titer
Weeks
2
4
6
8
10

FIGURE 1.2 Specificity, memory, and contraction of adaptive immune responses. Antigens X and Y activate different clones of B cells and induce the production of different antibodies (specificity). The secondary response to antigen X is more rapid and larger than the primary response (memory). Antibody levels decline with time after each immunization (contraction, the process that maintains homeostasis). The same features are seen in T cell–mediated immune responses.

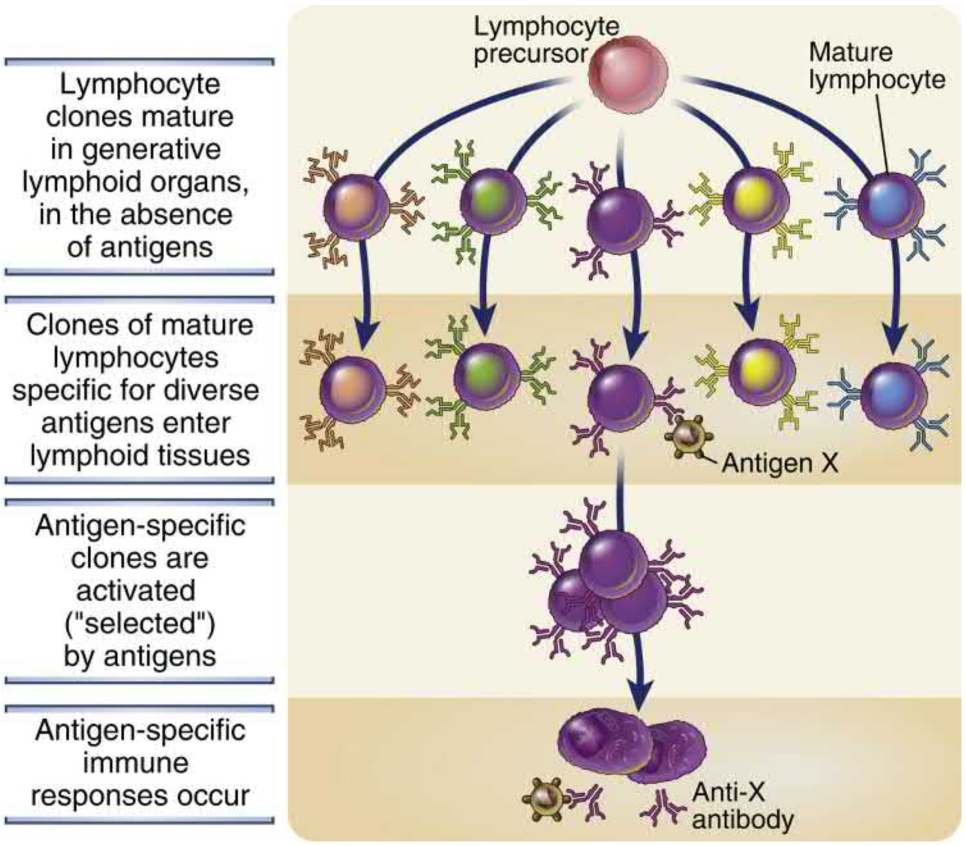

Lymphocyte clones mature in generative lymphoid organs, in the absence of antigens
Clones of mature lymphocytes specific for diverse antigens enter lymphoid tissues
Antigen-specific clones are activated ("selected") by antigens
Antigen-specific immune responses occur
Lymphocyte precursor
Mature lymphocyte
Antigen X
Anti-X antibody

FIGURE 1.3 Clonal selection.Each antigen *(X)* selects a preexisting clone of specific lymphocytes and stimulates the proliferation and differentiation of that clone. The diagram shows only B lymphocytes giving rise to antibody-secreting effector cells, but the same principle applies to T lymphocytes.

- ***Nonreactivity to self (self-tolerance).*** One of the most remarkable properties of every normal individual's immune system is its ability to recognize, respond to, and eliminate many foreign (nonself) antigens while not reacting harmfully to that individual's own (self) antigens. Immunologic unresponsiveness is also called **tolerance.** Tolerance to self antigens, or self-tolerance, is maintained by several mechanisms. These include eliminating lymphocytes that express receptors specific for some self antigens, inactivating self-reactive lymphocytes, or suppressing these cells by the actions of other (regulatory) cells. Abnormalities in the induction or maintenance of self-tolerance lead to immune responses against self (autologous) antigens, which may result in disorders called **autoimmune diseases.** The mechanisms of self-tolerance and its failure are discussed in Chapter 15.

In addition to these cardinal features of adaptive immunity, these responses have some other important properties.

- ***Because of the ability of lymphocytes and other immune cells to circulate among tissues, adaptive immunity is systemic,*** meaning that even if an immune response is initiated at one site it can provide protection at distant sites. This feature is, of course, essential for the success of vaccination—a vaccine administered in the subcutaneous or muscle tissue of the arm can protect from infections in any tissue.
- ***Immune responses are regulated by a system of positive feedback loops that amplify the reaction and by control mechanisms that prevent inappropriate or pathologic reactions.*** When lymphocytes are activated, they trigger mechanisms that further increase the magnitude of the response. This positive feedback is important to enable the small number of lymphocytes that are specific for any microbe to generate the large response needed to eradicate that infection. Many control mechanisms become active during immune responses, which prevent excessive activation of lymphocytes that could cause collateral damage to normal tissues, and also prevent responses against self antigens.

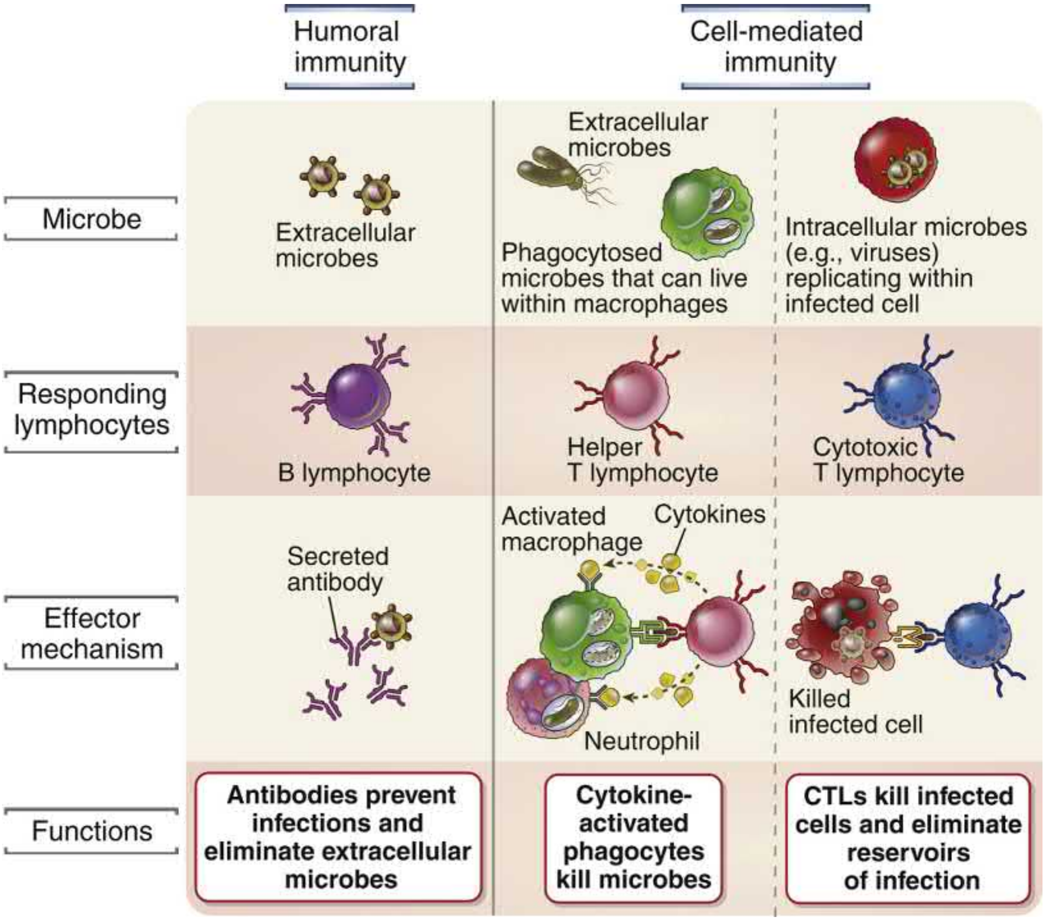

Humoral immunity
Cell-mediated immunity
Microbe
Responding lymphocytes
Effector mechanism
Functions
Extracellular microbes
Extracellular microbes
Phagocytosed microbes that can live within macrophages
Intracellular microbes (e.g., viruses) replicating within infected cell
B lymphocyte
Helper T lymphocyte
Cytotoxic T lymphocyte
Secreted antibody
Activated macrophage
Cytokines
Neutrophil
Killed infected cell
Antibodies prevent infections and eliminate extracellular microbes
Cytokine-activated phagocytes kill microbes
CTLs kill infected cells and eliminate reservoirs of infection

FIGURE 1.4 Types of adaptive immunity.In humoral immunity, B lymphocytes secrete antibodies that prevent infections and eliminate extracellular microbes. In cell-mediated immunity, helper T lymphocytes activate macrophages and neutrophils to kill phagocytosed microbes or cytotoxic T lymphocytes directly destroy infected cells.

Overview of Humoral and Cell-Mediated Immunity

There are two types of adaptive immunity, called humoral immunity and cell-mediated immunity, which are mediated by different types of lymphocytes and function to eliminate different types of microbes

(Figs. 1.4 and 1.5). **Humoral immunity** is mediated by molecules in the blood and mucosal secretions, called **antibodies,** which are produced by **B lymphocytes.** Antibodies recognize microbial antigens, neutralize the infectivity of the microbes, and target microbes for elimination by phagocytes and the complement system. Humoral immunity is the principal defense mechanism against microbes and their toxins located outside cells (e.g., in the lumens of the gastrointestinal and respiratory tracts and in the blood) because secreted antibodies can bind to these microbes and toxins, neutralize them, and assist in their elimination.

Cell-mediated immunity, also called cellular immunity, is mediated by **T lymphocytes**. Many microbes are ingested by but survive within phagocytes, and some microbes, notably viruses, infect and replicate in various host cells. In these locations the microbes are inaccessible to circulating antibodies. Defense against such infections is a function of cell-mediated immunity, which promotes the destruction of microbes inside phagocytes and the killing of infected cells to eliminate reservoirs of infection.

Different classes of lymphocytes may be distinguished by the expression of membrane proteins, many of which are designated by CD numbers. These surface molecules are also involved in the functions of the lymphocytes. We will introduce some of the surface molecules that are used to identify lymphocyte classes in Chapter 2 and discuss them further in later chapters. A summary of the molecules designated by CD numbers mentioned in the book is provided in Appendix I.

Protective immunity against a microbe may be provided either by the host's response to the microbe or by the transfer of antibodies that defend against the microbe (Fig. 1.6). The form of immunity that is induced by exposure to a foreign antigen is called **active immunity** because the immunized individual plays an active role in responding to the antigen. Individuals and lymphocytes that have not encountered a particular antigen are said to be naive, implying that they are immunologically inexperienced. Individuals who have responded to a microbial antigen and are protected from subsequent exposures to that microbe are said to be immune.

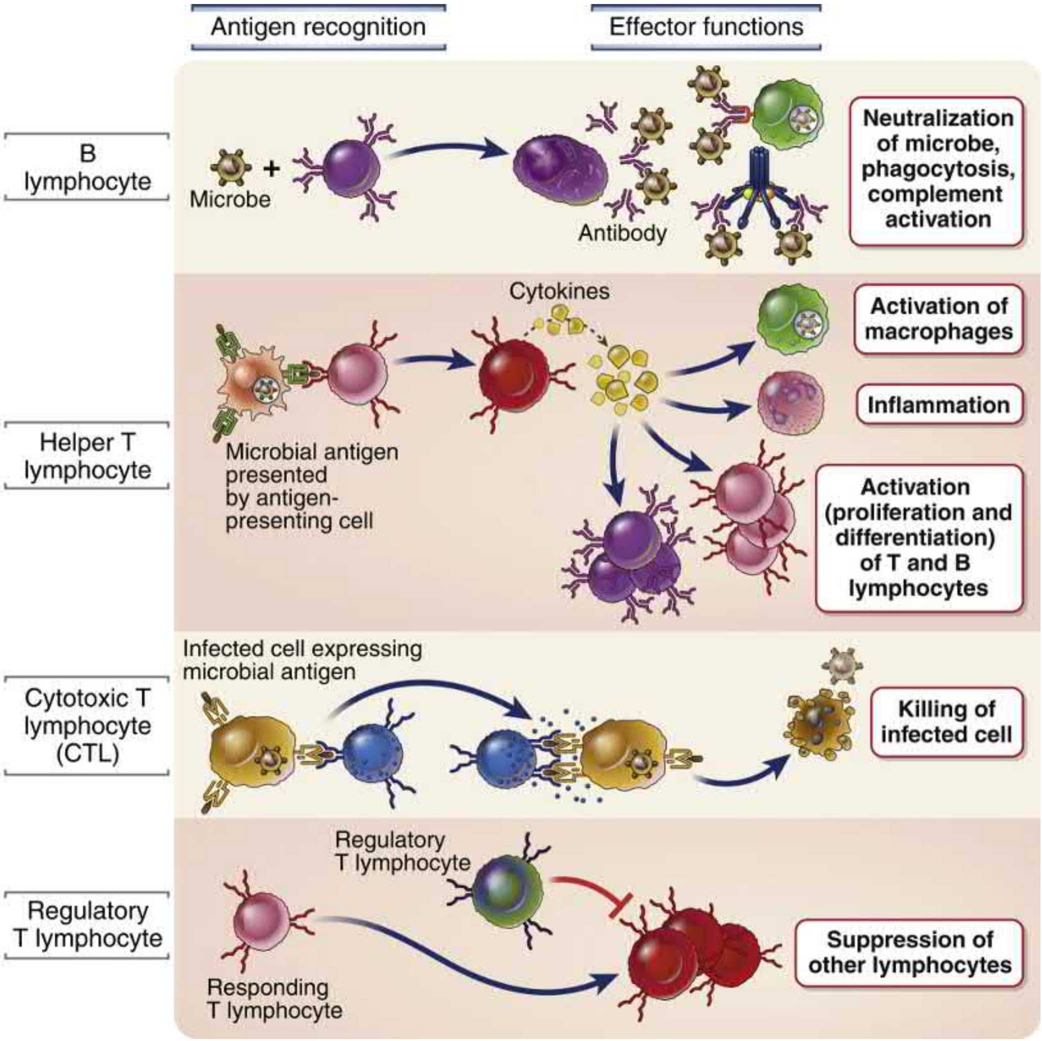

Antigen recognition
Effector functions
B lymphocyte
Microbe
Antibody
Neutralization of microbe, phagocytosis, complement activation
Helper T lymphocyte
Cytokines
Microbial antigen presented by antigen-presenting cell
Activation of macrophages
Inflammation
Activation (proliferation and differentiation) of T and B lymphocytes
Cytotoxic T lymphocyte (CTL)
Infected cell expressing microbial antigen
Killing of infected cell
Regulatory T lymphocyte
Regulatory T lymphocyte
Responding T lymphocyte
Suppression of other lymphocytes

FIGURE 1.5 Classes of lymphocytes.B lymphocytes recognize many different types of antigens and develop into antibody-secreting cells. Helper T lymphocytes recognize antigens on the surfaces of antigen-presenting cells and secrete cytokines, which stimulate different mechanisms of immunity and inflammation. Cytotoxic T lymphocytes recognize antigens in infected cells and kill these cells. Regulatory T cells suppress immune responses (e.g., to self antigens).

Immunity also can be conferred on an individual by transferring antibodies from an immunized individual into an individual who has not encountered the antigen (see Fig. 1.6). The recipient of such a transfer becomes immune to the particular antigen without ever having been exposed to or having responded to that antigen. Therefore, this form of immunity is called **passive immunity.** A physiologically important example of passive immunity is the transfer of maternal antibodies through the placenta to the fetus, which enables newborns to combat infections for several months before they develop the ability to produce antibodies themselves. Passive immunization is also a medically useful method for conferring resistance rapidly, without having to wait for an active immune response to develop. Passive immunization against potentially lethal toxins by the administration of antibodies from immunized animals or people is a lifesaving treatment for rabies infection and snake bites. Patients with some genetic immunodeficiency diseases are passively immunized by transfer of pooled antibodies from healthy donors.

The first demonstration of humoral immunity was provided by Emil von Behring and Shibasaburo Kitasato in 1890, using a passive immunization strategy. They showed that if serum from animals that had been immunized with an attenuated form of diphtheria toxin was transferred to naive animals, the recipients became specifically resistant to diphtheria infection. The active components of the serum were called antitoxins because they neutralized the pathologic effects of the diphtheria toxin. This result led to the treatment of otherwise lethal diphtheria infection by the administration of antitoxin, an achievement that was recognized by the award of the first Nobel Prize in Physiology or Medicine to von Behring. In the 1890s Paul Ehrlich postulated that immune cells use receptors, which he called side chains, to recognize microbial toxins and, subsequently, secrete these receptors to combat microbes. He coined the term antibodies (*antikörper* in German) for the serum proteins that bound foreign substances, such as toxins, and the substances that generated antibodies were called antigens. The modern definition of antigens includes molecules that bind to specific lymphocyte receptors, whether or not they stimulate immune responses. According to strict definitions, substances that stimulate immune responses are called immunogens, but the term antigen is often used interchangeably with immunogen. The properties of antibodies and antigens are described in Chapter 5. Ehrlich's concepts were a remarkably prescient model for the specificity of adaptive immunity. These early studies of antibodies led to the general acceptance of the humoral theory of immunity, according to which host defense against infections is mediated by substances present in body fluids (once called humors).

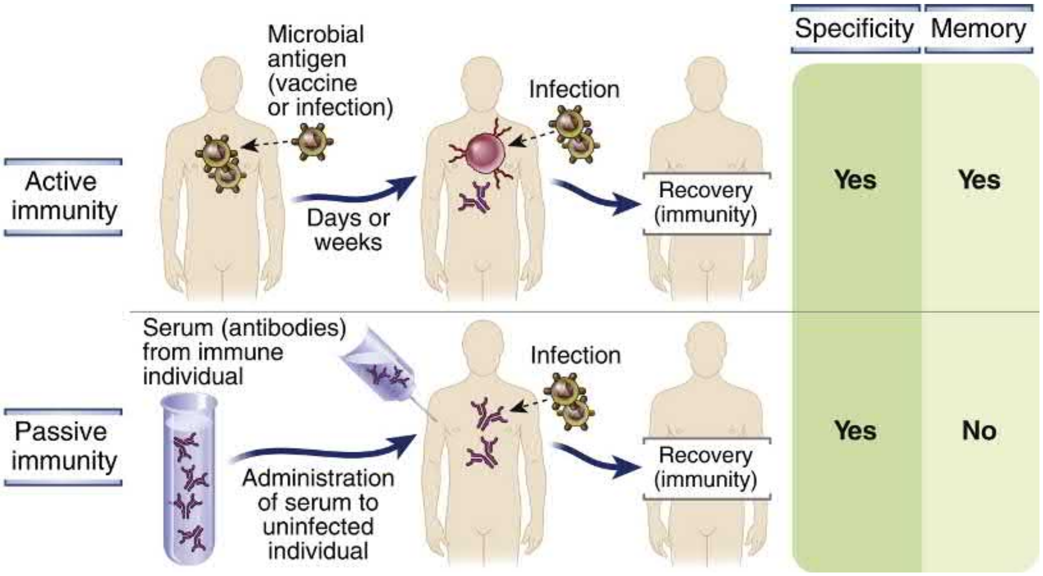

Microbial antigen (vaccine or infection)
Infection
Specificity
Memory
Active immunity
Days or weeks
Recovery (immunity)
Yes
Yes
Serum (antibodies) from immune individual
Infection
Passive immunity
Administration of serum to uninfected individual
Recovery (immunity)
Yes
No

FIGURE 1.6 Active and passive immunity.Active immunity is conferred by a host response to a microbe or microbial antigen, whereas passive immunity is conferred by adoptive transfer of antibodies or T lymphocytes specific for the microbe. Both forms of immunity provide resistance to infection and are specific for microbial antigens, but only active immune responses generate immunologic memory. Passive transfer of antibodies occurs during pregnancy (from mother to fetus), and injection of antibodies is used therapeutically to rapidly confer passive protective immunity against lethal toxins. Lymphocytes can be transferred only among genetically identical animals; in humans, lymphocytes from another individual would be recognized as foreign and rejected.

Ilya Metchnikoff initially championed the cellular theory of immunity, which stated that host cells are the principal mediators of immunity. His demonstration of phagocytes surrounding a thorn stuck into a translucent starfish larva, published in 1883, was perhaps the first experimental evidence that cells respond to foreign invaders. Ehrlich and Metchnikoff shared the Nobel Prize in 1908, in recognition of their contributions to establishing these fundamental principles of immunity. Sir Almroth Wright's observation in the early 1900s that factors in immune serum enhanced the phagocytosis of bacteria by coating the bacteria, a process known as **opsonization,** lent support to the belief that antibodies prepare microbes for ingestion by phagocytes. These early cellularists were unable to prove that specific immunity to microbes could be mediated by cells. The importance of cellular immunity in host defense became firmly established in the 1950s, when it was shown that resistance to an intracellular bacterium, *Listeria monocytogenes,* could be transferred to animals with cells but not with serum. We now know that the specificity of cell-mediated immunity is due to T lymphocytes, which often function in concert with other cells, such as phagocytes, to eliminate microbes.

In the clinical setting, immunity to a previously encountered microbe is measured indirectly, either by assaying for the presence of products of immune responses (such as serum antibodies specific for microbial antigens) or by administering substances purified from the microbe and measuring reactions to these substances. A reaction to an antigen is detectable only in individuals who have previously encountered the antigen, reflecting memory for that antigen. These individuals are said to be sensitized to the antigen, and the reaction is an indication of sensitivity. Such a reaction to a microbial antigen implies that the sensitized individual is capable of mounting a protective immune response to the microbe.

Initiation and Development of Adaptive Immune Responses

Adaptive immune responses develop in several steps, starting with the capture of antigen, followed by the activation of specific lymphocytes (Fig. 1.7).

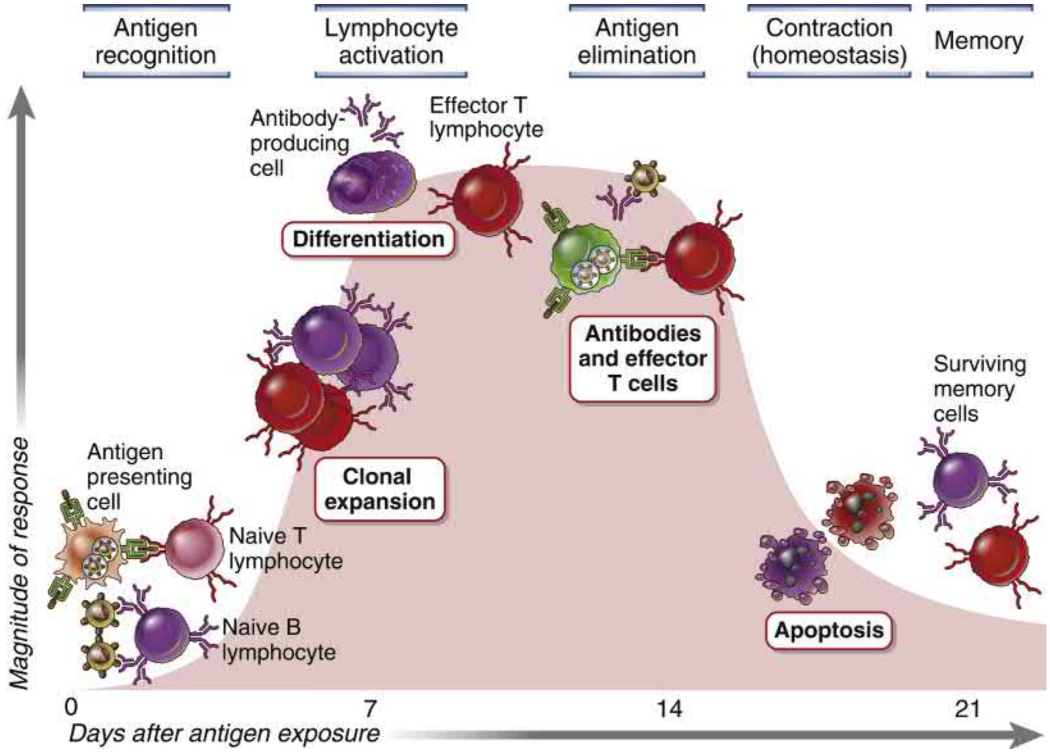

Antigen recognition
Lymphocyte activation
Antigen elimination
Contraction (homeostasis)
Memory
Antibody-producing cell
Effector T lymphocyte
Differentiation
Antibodies and effector T cells
Surviving memory cells
Clonal expansion
Antigen presenting cell
Naive T lymphocyte
Naive B lymphocyte
Apoptosis
Magnitude of response
0
7
14
21
Days after antigen exposure

FIGURE 1.7 Development of adaptive immune responses.Adaptive immune responses consist of distinct steps, the first three being the recognition of antigen, the activation of lymphocytes, and the elimination of antigen (the effector phase). The response contracts (declines) as antigen-stimulated lymphocytes die by apoptosis, restoring homeostasis, and the antigen-specific cells that survive are responsible for memory. The duration of each phase may vary in different immune responses. The *y*-axis represents an arbitrary measure of the magnitude of the response. These principles apply to humoral immunity (mediated by B lymphocytes) and cell-mediated immunity (mediated by T lymphocytes).

Most microbes and other antigens enter through epithelial barriers and colonize tissues, and adaptive immune responses to these antigens develop in secondary (peripheral) lymphoid organs. The initiation of adaptive immune responses requires that antigens be captured and displayed to specific lymphocytes. The cells that serve this role are called **antigen-presenting cells** (APCs). The most specialized APCs are **dendritic cells** (DCs), which capture microbial antigens that enter from the external environment, transport these antigens to lymphoid organs, and present the antigens to naive T lymphocytes to initiate immune responses. Other cell types function as APCs at different stages of cell-mediated and humoral immune responses. We will describe the functions of APCs in Chapter 6.

Naive lymphocytes express antigen receptors but have not responded to antigen. The activation of these lymphocytes by antigen leads to the proliferation of these cells, resulting in an increase in the size of the antigen-specific clones, called **clonal expansion**. This is followed by differentiation of the activated lymphocytes into cells capable of eliminating the antigen, called **effector cells** because they mediate the ultimate effect of the immune response, and **memory cells** that survive for long periods and mount strong responses to repeat antigen encounter. Antigen elimination often requires the participation of other, nonlymphoid cells, such as macrophages and neutrophils, which are also sometimes called effector cells. These steps in lymphocyte activation and differentiation into effector cells typically take a few days, which explains why the adaptive response is slow to develop and innate immunity has to provide protection initially.

After the adaptive immune response has eradicated the infection, the stimuli for lymphocyte activation dissipate and most of the effector cells die, resulting in the decline of the response. Memory cells remain, ready to respond vigorously if the same infection recurs.

The cells of the immune system interact with one another and with other host cells via secreted proteins called cytokines. Such interactions are essential during both the initiation and effector stages of innate and adaptive immune responses. **Cytokines** are a large group of secreted proteins with diverse structures and functions, which regulate and coordinate many activities of the cells of innate and adaptive immunity. All cells of the immune system secrete at least some cytokines and express specific signaling receptors for several cytokines. Among the many functions of cytokines we will discuss throughout this book are promoting the growth and differentiation of immune cells, activating the functions of lymphocytes and phagocytes that eliminate microbes (called effector functions), and stimulating directed movement of immune cells from blood into tissues and within tissues. A large subset of structurally related cytokines that regulate cell adhesion and migration are called **chemokines.** Cytokines also are involved in immunological diseases, and some of the most effective drugs developed to treat these diseases target cytokines. We will describe the functions of individual cytokines when we discuss immune responses in which these proteins play important roles. A list of cytokines and a brief summary of their properties are provided in Appendix II.

Humoral Immunity

B lymphocytes that recognize antigens proliferate and differentiate into plasma cells that secrete different classes of antibodies with distinct functions. Each clone of B cells expresses a cell surface antigen receptor, which is a membrane-bound form of antibody, with a unique antigen specificity. Many different types of antigens, including proteins, polysaccharides, lipids, and small molecules, are capable of eliciting antibody responses. The response of B cells to protein antigens requires activating signals (help) from $CD4^+$ T cells (which is the historical reason for calling these T cells helper cells). B cells can respond to many nonprotein antigens without the participation of helper T cells. Each plasma cell secretes antibodies that have the same antigen-binding site as the B cell surface antigen receptor that first recognized the antigen. Polysaccharides and lipids stimulate secretion mainly of the antibody class (isotype) called immunoglobulin M (IgM). Protein antigens induce the production of antibodies of different classes (IgG, IgA, IgE) from a single clone of B cells, a process called heavy-chain class (or isotype) switching. These different antibody classes serve distinct functions, mentioned later. Helper T cells also stimulate the production of antibodies with increased affinity for the antigen. This process, called affinity maturation, improves the quality of the humoral immune response.

The humoral immune response combats microbes in many ways. Antibodies bind to microbes and prevent them from infecting cells, thus neutralizing the microbes. Antibody-mediated neutralization is the only mechanism of adaptive immunity that stops an infection before it is established; this is why eliciting the production of potent neutralizing antibodies is a key goal of vaccination. IgG antibodies coat microbes and target them for phagocytosis because phagocytes (neutrophils and macrophages) express receptors for parts of IgG molecules. IgG and IgM activate the complement system, and complement products promote phagocytosis and destruction of microbes. IgA is secreted from mucosal epithelia and neutralizes microbes in the lumens of mucosal tissues, such as the respiratory and gastrointestinal tracts, thus preventing inhaled and ingested microbes from infecting the host. Maternal IgG is actively transported across the placenta and protects the newborn until the baby's immune system becomes mature. Most IgG antibodies have half-lives in the circulation of approximately 3 weeks, whereas other classes of antibodies have half-lives of just a few days. Some antibody-secreting plasma cells migrate to the bone marrow or mucosal tissues and live for years, continuing to produce low levels of antibodies. The antibodies that are secreted by these long-lived plasma cells provide immediate protection if the microbe returns to infect the individual. More effective protection is provided by memory cells that are activated by the microbe and rapidly differentiate to generate large numbers of plasma cells.

Cell-Mediated Immunity

T lymphocytes, the cells of cell-mediated immunity, recognize the antigens of cell-associated microbes, and different types of T cells help phagocytes to destroy these microbes or kill the infected cells. T cells do not produce antibody molecules. Their antigen receptors are membrane molecules distinct from but structurally related to antibodies (see Chapter 7). T lymphocytes have a restricted specificity for antigens; they recognize peptides derived from foreign proteins that are bound to host proteins called **major histocompatibility complex** (MHC) molecules, which are expressed on the surfaces of other cells. As a result, these T cells recognize and respond to cell-associated but not soluble antigens (see Chapter 6).

T lymphocytes consist of functionally distinct populations, the best defined of which are **helper T cells** and **cytotoxic** (or **cytolytic**) **T lymphocytes** (CTLs). Helper T cells function mainly by secreted cytokines and membrane molecules, which activate other cells to kill microbes, whereas CTLs produce molecules that directly kill infected host cells. Some T lymphocytes, which are called **regulatory T cells,** function mainly to inhibit immune responses. We will return to a more detailed discussion of the properties of lymphocytes in Chapter 2 and in later chapters.

Upon activation in secondary lymphoid organs, naive T lymphocytes differentiate into effector cells, and many of them leave the lymphoid organs and migrate to sites of infection. When these effector T cells again encounter cell-associated microbes, they are activated to perform the functions that are responsible for elimination of the microbes. Cytokines produced by $CD4^+$ helper T cells recruit leukocytes, and both cytokines and plasma membrane proteins stimulate production of microbicidal substances in phagocytes. Thus, these T cells help phagocytes to kill the infectious pathogens. Other $CD4^+$ helper T cells secrete cytokines that help B cells to produce a type of antibody called IgE and activate leukocytes called eosinophils, which are able to kill helminths that may be too large to be phagocytosed. Some $CD4^+$ helper T cells stay in the lymphoid organs and use membrane molecules and cytokines to stimulate B cells to make highly effective and functionally specialized antibodies.

$CD8^+$ CTLs kill cells harboring microbes in the cytoplasm. These microbes may be viruses that infect many cell types or bacteria that are ingested by macrophages but escape from phagocytic vesicles into the cytoplasm (where they are inaccessible to the killing machinery of phagocytes, which is largely confined to vesicles). By destroying the infected cells, CTLs eliminate the reservoirs of infection. CTLs also kill tumor cells that express antigens that are recognized as foreign.

In the remainder of the book, we describe in detail the recognition, activation, regulation, and effector phases of innate and adaptive immune responses. The principles introduced in this chapter recur throughout this book.

Summary

- Protective immunity against microbes is mediated by the early reactions of innate immunity and the later responses of adaptive immunity. Innate immune responses are stimulated by molecular structures shared by groups of microbes and by molecules expressed by damaged host cells. Adaptive immunity is specific for different microbial and nonmicrobial antigens and is increased by repeated exposures to antigen (immunologic memory).
- Many features of adaptive immunity are of fundamental importance for its normal functions. These include specificity for different antigens, a diverse repertoire capable of recognizing a wide variety of antigens, memory of antigen exposure, and the ability to discriminate between foreign antigens and self antigens.
- Immunity may be acquired by a response to antigens (active immunity) or conferred by transfer of antibodies or effector cells (passive immunity).
- Lymphocytes are the only cells capable of specifically recognizing antigens and are thus the principal cells of adaptive immunity. The total population of lymphocytes consists of many clones, each with a unique antigen receptor and specificity. The two major subsets of

lymphocytes are B cells and T cells, and they differ in their antigen receptors and functions.

- The adaptive immune response is initiated by the recognition of foreign antigens by specific lymphocytes. Specialized antigen-presenting cells capture microbial antigens and display these antigens for recognition by lymphocytes. Lymphocytes respond by proliferating and by differentiating into effector cells, whose function is to eliminate the antigen, and into memory cells, which show enhanced responses on subsequent encounters with the antigen. The elimination of antigens often requires the participation of various effector cells.
- Humoral immunity is mediated by antibodies secreted by B lymphocytes and their differentiated progeny, plasma cells, and is the mechanism of defense against extracellular microbes. Antibodies neutralize the infectivity of microbes and promote the elimination of microbes by phagocytes and by activation of the complement system.
- Cell-mediated immunity is mediated by T lymphocytes and their products, such as cytokines, and is important for defense against intracellular microbes. $CD4^+$ helper T lymphocytes help macrophages to eliminate ingested microbes and help B cells to produce antibodies. $CD8^+$ cytotoxic T lymphocytes kill cells harboring intracellular pathogens, thus eliminating reservoirs of infection.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

Historical Ideas

∗Burnet FM. A modification of Jerne's theory of antibody production using the concept of clonal selection. *Aust J Sci*. 1957;20:67–69. (*A description of the clonal selection theory. Burnet received the Nobel Prize for his contributions to the understanding of immune recognition of self vs nonself. See https://www.nobelprize.org/prizes/medicine/1960/burnet/speech/* .)

Cohn M, Mitchison N.A, Paul W.E, et al. Reflections on the clonal-selection theory. *Nat Rev Immunol* . 2007;7:823–830.

∗Ehrlich P. Croonian lecture: on immunity with special reference to cell life. *Proc Royal Soc Lond*. 1900. Also Ehrlich, P., Nobel lecture: partial cell functions. *(Ehrlich's side-chain theory was the first idea about specific antigen receptors in the immune system. See http://nobelprize.org/nobel_prizes/medicine/laureates/1908/ehrlich%5dlecture.pdf* .)

Jerne N.K. The natural-selection theory of antibody formation. *Proc Natl Acad Sci U S A* . 1955;41:849–857.

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Silverstein A.M. Cellular versus humoral immunology: a century-long dispute. *Nat Immunol* . 2003;4:425–428.

Turk J.L. Almroth Wright: phagocytosis and opsonization. *J Roy Soc Med* . 1994;87:576–577.

∗von Behring E. Nobel lecture: serum therapy in therapeutics and medical science. *(The discovery of passive immunization with antibodies for the treatment of diphtheria and other infectious diseases. See https://www.nobelprize.org/prizes/medicine/1901/behring/lecture.*)

∗Wright AE. *Studies on Immunisation*. London: Constable; 1909. See also Turk JL. Almroth Wright: phagocytosis and opsonization. *J Roy Soc Med*. 1994;87:576-577. (*The discovery of antibody-mediated opsonization for phagocytosis. Wright's friendship with the playwright George Bernard Shaw led to Shaw modeling a main character in his play The Doctor's Dilemma after Wright and attributing to him the proposed treatment for disease—"Stimulate the phagocytes!"*)

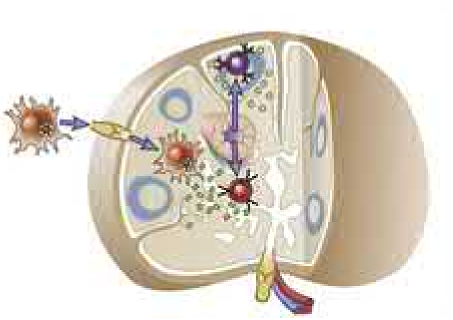
Evolution of the Immune System

Boehm T, Swann J.B. Origin and evolution of adaptive immunity. *Annu Rev Anim Biosci* . 2014;2:259–283.

Flajnik M.F. A cold-blooded view of adaptive immunity. *Nat Rev Immunol* . 2018;18:438–453.

Litman G.W, Rast J.P, Fugmann S.D. The origins of vertebrate adaptive immunity. *Nat Rev Immunol* . 2010;10:543–553.

Chapter 2: Cells and Tissues of the Immune System



Cells of the Immune System,
  - Phagocytes,
  - Mast Cells, Basophils, and Eosinophils,
  - Dendritic Cells (DCs),
  - Lymphocytes,
  - Natural Killer Cells and Cytokine-Secreting Innate Lymphoid Cells,

Anatomy and Functions of Lymphoid Tissues,
  - Bone Marrow,
  - Thymus,
  - Lymphatic System,
  - Lymph Nodes,
  - Spleen,
  - Cutaneous and Mucosal Immune Systems,

Summary,

The cells of the innate and adaptive immune system are normally present as circulating cells in the blood and lymph and as extravascular cells in lymphoid organs and scattered in virtually all tissues. The anatomic arrangement of these cells in lymphoid tissues and their ability to circulate and exchange among blood, lymph, and tissues are of critical importance for immune responses. The immune system faces numerous challenges to generate effective protective responses against infectious pathogens. First, the system must be able to respond rapidly to small numbers of many different microbes that may be introduced at any site in the body. Second, in the adaptive immune response, there are very few naive lymphocytes that can specifically recognize and respond to any one antigen. Third, the effector mechanisms of the adaptive immune system (antibodies and effector T cells) may have to locate and destroy microbes at sites that are distant from the site where the immune response was induced. The capacity of the immune system to meet these challenges and to optimally perform its protective functions is dependent on the remarkably rapid and varied responses of immune cells, the way these cells are organized in lymphoid tissues, and their ability to migrate from one tissue to another.

This chapter describes the cells and tissues that make up the immune system. In Chapter 3, we describe the traffic patterns of lymphocytes throughout the body and the mechanisms of migration of lymphocytes and other leukocytes.

Cells of the Immune System

The cells that serve specialized roles in innate and adaptive immune responses are phagocytes, dendritic cells (DCs), antigen-specific lymphocytes, and various other leukocytes that function to eliminate antigens. These cells were introduced briefly in Chapter 1. These cells are almost all derived from hematopoietic stem cells (HSCs) in the bone marrow, which differentiate along branching lineages. Based on their common precursors, immune cells are broadly classified as either **myeloid cells,** which include phagocytes and most DCs, or **lymphoid cells**, which include all lymphocytes. The numbers of some of these cell types in the blood are listed in Table 2.1. Although most of these cells are found in the blood, the responses of lymphocytes to antigens usually occur in lymphoid and other tissues and therefore may not be reflected by changes in the numbers of blood lymphocytes.

The expression of various membrane proteins is used to distinguish distinct populations of cells in the immune system. For instance, most helper T cells express a surface protein called CD4, and most cytotoxic T lymphocytes (CTLs) express a different surface protein called CD8. These and many other surface proteins are often called markers because they identify and discriminate between (mark) different cell populations. The function of most of these markers in the cell on which they are expressed is now understood. The most common way to determine if a particular marker is expressed on a cell is to test if antibodies specific for the marker bind to the cell. In this context, the antibodies are used by investigators or clinicians as analytical tools. Hundreds of different pure antibody preparations, called monoclonal antibodies, are available, each specific for a different molecule and labeled with chemicals that can be readily detected on cell surfaces by use of appropriate instruments. (Monoclonal antibodies are described in Chapter 5, and methods to detect labeled antibodies bound to cells are discussed in Appendix III.) The cluster of differentiation (CD) nomenclature is a widely adopted uniform method for naming cell surface molecules that are sometimes characteristic of a particular cell lineage or differentiation stage, and are recognized by a group (cluster) of monoclonal antibodies. Thus, all antigenically distinguishable cell surface proteins and some carbohydrates are given a CD number designation (e.g., CD1, CD2). Although originally devised to define circulating immune cell (leukocyte) subtypes, CD markers are found on all cell types in the body. Cell surface molecules (now identified by CD numbers) have important roles in immune responses and are the targets of many therapeutic antibodies used in the treatment of inflammatory diseases and cancer. Appendix I provides a current list of leukocyte CD markers that are mentioned in this book.

TABLE 2.1

Normal Blood Cell Counts

	Mean Number (per mm^3)	Normal Range
White blood cells (leukocytes)	7400	4500–11,000/mm^3
Neutrophils	4400	40%–60%
Eosinophils	200	1%–4%
Basophils	40	<1%
Lymphocytes	2500	20%–40%
Monocytes	300	2%–8%

Phagocytes

Phagocytes, including neutrophils and macrophages, are cells whose primary function is to ingest and destroy microbes and remove damaged tissues. The functional responses of phagocytes in host defense consist of sequential steps: recruitment of the cells to the sites of infection, recognition of and activation by microbes, ingestion of the microbes by the process of phagocytosis, and destruction of ingested microbes. In addition, through direct contact and by secreting cytokines, phagocytes communicate with other cells in ways that promote or regulate immune responses.

Blood neutrophils and monocytes, which differentiate into macrophages after entering tissues, are produced in the bone marrow, circulate in the blood, and are recruited to sites of inflammation. Although both are actively phagocytic, they differ in significant ways (Table 2.2). The neutrophil response is more rapid and the lifespan of these cells after they enter tissues is short, whereas macrophages in tissues can live for long periods so that the macrophage response may last for a prolonged time. Neutrophils mainly use cytoskeletal rearrangements and enzyme activation to mount rapid, transient responses, whereas macrophage responses rely more on induced gene transcription and protein expression. In addition, as we discuss later, there are subsets of macrophages that normally reside in healthy tissues, but neutrophils do not. The functions of phagocytes are important in innate immunity (see Chapter 4) and also in the effector phase of some adaptive immune responses (see Chapter 10). As a prelude to more detailed discussions of the role of phagocytes in immune responses in later chapters, here we will describe the development and morphologic features of neutrophils and macrophages and briefly introduce their functional responses.

TABLE 2.2

	Neutrophils	**Macrophages**
Origin	HSCs in bone marrow	Blood monocytes: derived from HSCs in bone marrow (in inflammatory reactions) Many tissue-resident macrophages: derived from stem cells in yolk sac or fetal liver (early in development)
Life span in tissues	1–2 days	Inflammatory macrophages: days or weeks Tissue-resident macrophages: years
Responses to activating stimuli	Rapid, short-lived, activation of preformed enzymes	More prolonged, slower, often dependent on new gene transcription
Phagocytosis	Rapid ingestion of microbes	Prolonged ability to ingest microbes, apoptotic cells, tissue debris, foreign material
Reactive oxygen species	Rapidly induced by assembly of phagocyte oxidase (respiratory burst)	Less prominent
Nitric oxide	Low levels or none	Induced after transcriptional activation of iNOS
Degranulation	Major response; induced by cytoskeletal rearrangement	Not prominent
Cytokine production	Low levels per cell	Major functional activity, large amounts per cell, requires transcriptional activation of cytokine genes
NET formation	Rapidly induced, by extrusion of nuclear contents	No
Pyroptosis	No	Prominent: caspase-1 activation

This table lists the major differences between neutrophils and macrophages. The reactions summarized above are described in the text. Note that the two cell types share many features, such as phagocytosis, chemotaxis, and ability to migrate through blood vessels into tissues.

HSC, Hematopoietic stem cells; *iNOS,* inducible nitric oxide synthase; *NET,* neutrophil extracellular trap.

Neutrophils

Neutrophils are the most abundant population of circulating white blood cells and the principal cell type in acute inflammatory reactions. Neutrophils circulate as spherical cells approximately 12 to 15 μm in diameter with numerous membranous projections. The nucleus is segmented into three to five connected lobules (Fig. 2.1A). Because of their nuclear morphology, neutrophils are also called polymorphonuclear leukocytes (PMNs), to contrast them with mononuclear cells (macrophages and lymphocytes), whose nuclei are not multilobed. The cytoplasm contains two types of membrane-bound granules. The majority of these granules, called specific granules, are filled with enzymes, such as lysozyme, collagenase, and elastase. These granules do not stain strongly with either basic or acidic dyes (hematoxylin and eosin, respectively), which distinguishes neutrophils from two other types of circulating leukocytes with cytoplasmic granules, called **basophils** and **eosinophils.** The remainder of the granules of neutrophils, called azurophilic granules because they are stained by azure A dyes, contain enzymes (e.g., myeloperoxidase) and microbicidal substances, including defensins and cathelicidins, which we will discuss in Chapter 4. Neutrophils are produced in the bone marrow and arise from precursors that also give rise to circulating monocytes. Production of neutrophils is stimulated by granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). An adult human produces more than 1 × 10^{11} neutrophils per day, each of which circulates in the blood for a few hours or up to 5 days before dying. Neutrophils may migrate to sites of infection rapidly after the entry of microbes. After entering tissues, neutrophils function for only 1 to 2 days and most of them then die.

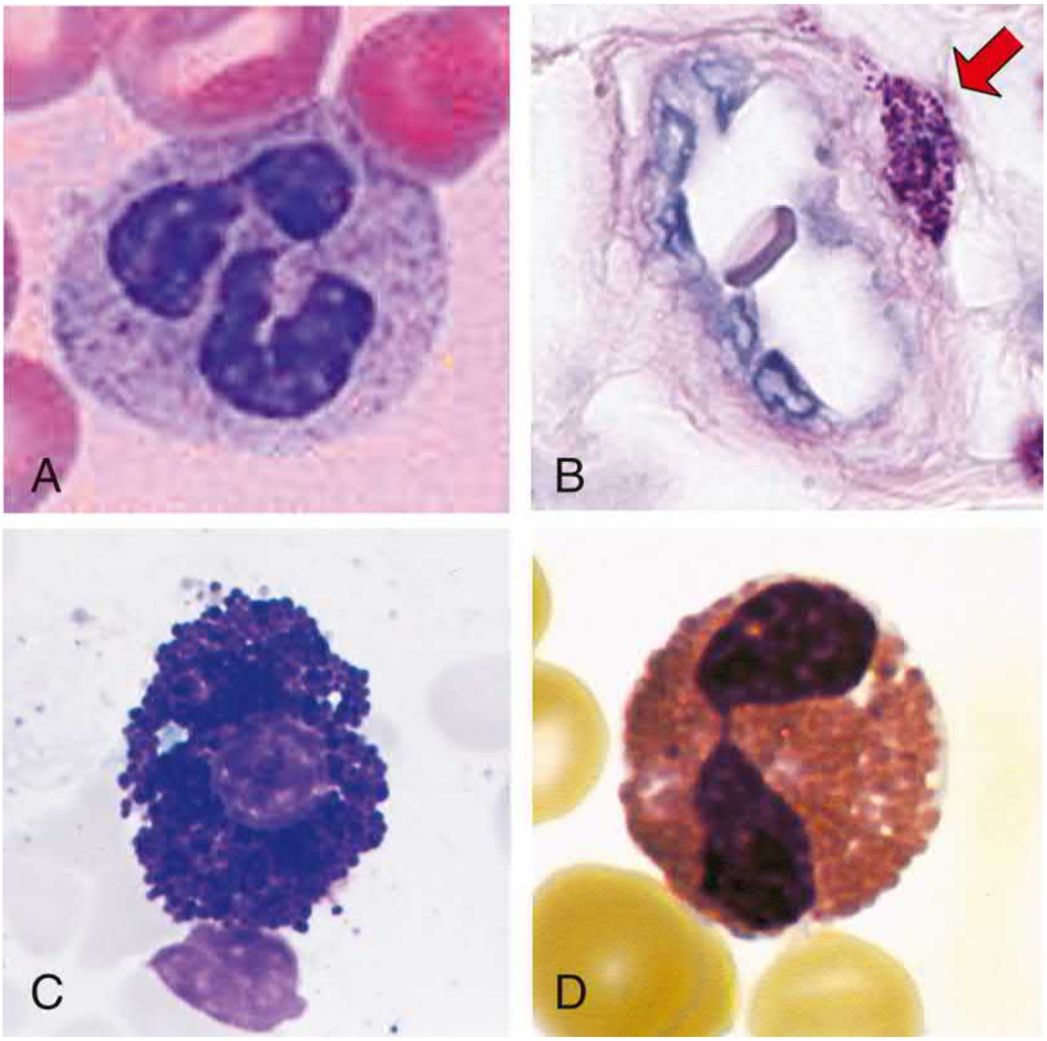


FIGURE 2.1 Morphology of neutrophils, mast cells, basophils, and eosinophils. **A,** The light micrograph of a Wright-Giemsa–stained blood neutrophil shows the multilobed nucleus, because of which these cells are also called polymorphonuclear leukocytes, and the faint cytoplasmic granules. **B,** The light micrograph of a Wright-Giemsa–stained section of skin shows a mast cell *(arrow)* adjacent to a small blood vessel, identifiable by the red blood cell in the lumen. The cytoplasmic granules in the mast cell, which are stained purple, are filled with histamine and other mediators that act on adjacent blood vessels to promote increased blood flow and delivery of

plasma proteins and leukocytes into the tissue. **C,** The light micrograph of a Wright-Giemsa–stained blood basophil shows the characteristic blue-staining cytoplasmic granules. **D,** The light micrograph of a Wright-Giemsa–stained blood eosinophil shows the characteristic segmented nucleus and *red* staining of the cytoplasmic granules.

B, Courtesy of Dr. George Murphy, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts. *C,* Courtesy of Dr. Jonathan Hecht, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.

The major function of neutrophils is to phagocytose microbes, especially opsonized microbes, and products of necrotic cells and destroy these in phagolysosomes. In addition, neutrophils may secrete granule contents and also extrude their nuclear contents, forming neutrophil extracellular traps (NETs), which serve to immobilize and kill extracellular microbes but also may damage healthy tissues.

Mononuclear Phagocytes

The mononuclear phagocyte system includes circulating bone marrow–derived cells called monocytes, many of which become macrophages when they migrate into tissues, and tissue-resident macrophages, which are initially derived from yolk sac or hematopoietic precursors during fetal life.

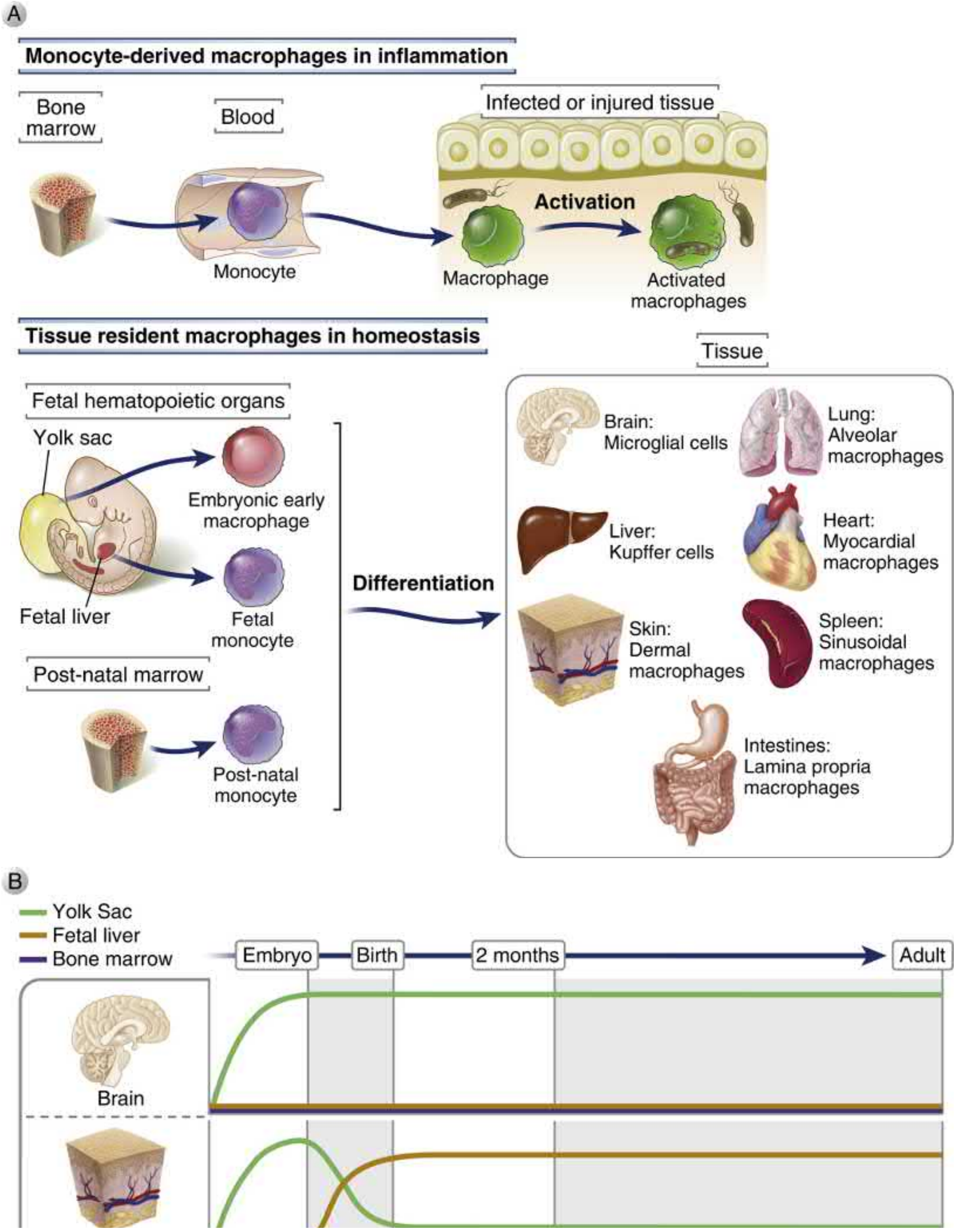
Development of Macrophages and Monocytes

After birth, cells of the monocyte-macrophage lineage arise from committed precursor cells in the bone marrow, driven by a cytokine called monocyte (or macrophage) colony-stimulating factor (M-CSF). These precursors mature into monocytes, which enter and circulate in the blood (Fig. 2.2), where they have a short life span of approximately 1 to 7 days. Blood monocytes are efficiently recruited into tissue sites of infection or injury, and therefore most macrophages at sites of inflammation are monocyte-derived.

Most long-lived tissue-resident macrophages are derived not from the bone marrow but from yolk sac or fetal liver precursors during fetal development. These cells have self-renewal capacity, so they can maintain stable numbers. They often assume specialized phenotypes depending on the organ (see Fig. 2.2). Examples are Kupffer cells lining the sinusoids in the liver, alveolar macrophages in the lung, and microglial cells in the brain. In the steady state, blood monocytes are recruited at a low rate into healthy tissues, where they differentiate into tissue-resident macrophages. This pathway of monocyte differentiation into tissue macrophages supplements the self-renewal of the fetally derived cells, and accounts for varying fractions of resident macrophages in different tissues.

Subsets of Monocytes

Monocytes are 10 to 15 μm in diameter, and they have bean-shaped nuclei and finely granular cytoplasm containing lysosomes, phagocytic vacuoles, and cytoskeletal filaments (Fig. 2.3). All human monocytes express class II major histocompatibility complex (MHC) molecules, CD11b, and CD86, and all mouse monocytes express CD115, CD11b, and CD64. However, monocytes are heterogeneous and consist of different subsets distinguishable by cell surface markers and functions but not by morphology. In both humans and mice, the most numerous monocytes, called classical or inflammatory monocytes, (comprising 90% to 95% of blood monocytes in humans) produce inflammatory mediators, are phagocytic, and are rapidly recruited to sites of infection or tissue injury. The second type of circulating monocyte, called nonclassical monocytes (5% to 10% of blood monocytes), are recruited into tissues after infection or injury and may contribute to repair. Classical/inflammatory monocytes are most often distinguished from nonclassical monocytes by their relatively high expression of CD14 (human) or Ly6C and CCR2 (mice). Some nonclassical monocytes are known to crawl along endothelial surfaces (described as patrolling), where they scavenge luminal microparticles and may play a role in eliminating circulating microbes and in repairing endothelial barrier defects. The developmental relationship between monocyte subsets is not fully understood.


A
Monocyte-derived macrophages in inflammation
Bone marrow
Blood
Infected or injured tissue
Activation
Monocyte
Macrophage
Activated macrophages
Tissue resident macrophages in homeostasis
Tissue
Fetal hematopoietic organs
Yolk sac
Embryonic early macrophage
Fetal liver
Fetal monocyte
Post-natal marrow
Post-natal monocyte
Differentiation
Brain: Microglial cells
Lung: Alveolar macrophages
Liver: Kupffer cells
Heart: Myocardial macrophages
Skin: Dermal macrophages
Spleen: Sinusoidal macrophages
Intestines: Lamina propria macrophages
B
Yolk Sac
Fetal liver
Bone marrow
Embryo
Birth
2 months
Adult
Brain

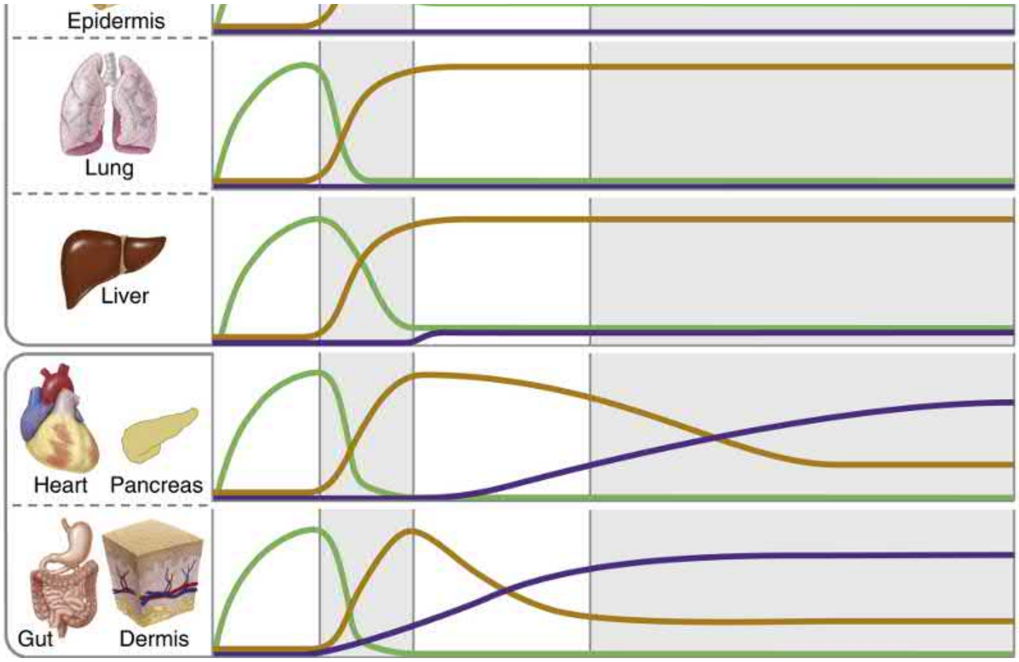


FIGURE 2.2 Maturation of mononuclear phagocytes. **A,** Pathways of macrophage development. During inflammatory reactions, precursors in the bone marrow give rise to circulating monocytes, which enter peripheral tissues and mature to form short-lived macrophages, which are activated locally. Many tissue-resident macrophages develop in fetal life from primitive hematopoietic precursors in the yolk sac and hematopoietic precursors in fetal liver and bone marrow. Blood monocytes may contribute to the tissue-resident pool of macrophages in postnatal life, to varying degrees between different tissues. **B,** The relative contributions of precursors from the yolk sac, fetal liver, and postnatal bone marrow to macrophages resident in different tissues in the steady state, as determined by cell fate mapping studies in mice.

B, Courtesy of Florent Ginhoux and Svetoslav Chakarov. Modified from Ginhoux F, Guillams M. Tissue-resident macrophage ontogeny and homeostasis. *Immunity.* 2016;44:439-449, with permission of the publisher.

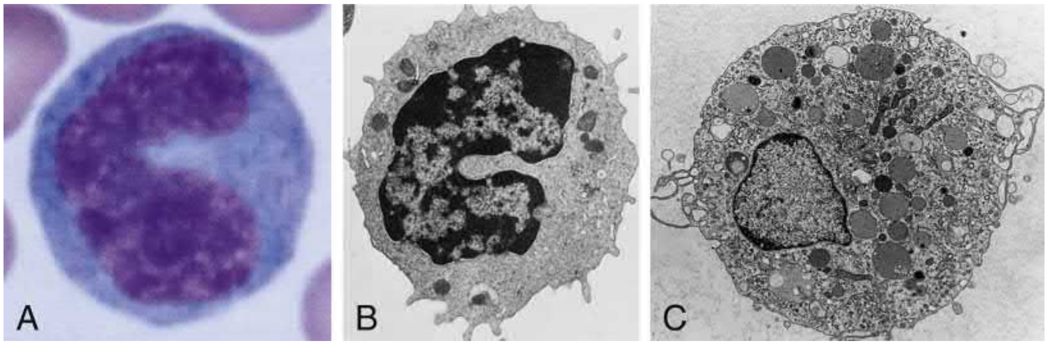


FIGURE 2.3 Morphology of mononuclear phagocytes. **A,** Light micrograph of a monocyte in a peripheral blood smear. **B,** Electron micrograph of a peripheral blood monocyte. **C,** Electron micrograph of an activated tissue macrophage showing numerous phagocytic vacuoles and cytoplasmic organelles.

Courtesy of Dr. Noel Weidner, Department of Pathology, University of California, San Diego, California.

Functions of Macrophages

Macrophages play crucial roles in innate and adaptive immune responses to infections and in repair of damaged tissues (Fig. 2.4).

- A major function of monocyte-derived macrophages in host defense is to ingest microbes by the process of phagocytosis and then to kill the ingested microbes. The mechanisms of phagocytosis and killing, which we will discuss in Chapter 4, include formation of cytoplasmic membrane–bound organelles that contain the microbes, the fusion of these organelles with lysosomes, the enzymatic generation of reactive oxygen and nitrogen species in the lysosome that are toxic to microbes, and the digestion of microbial proteins by proteolytic enzymes.

- Tissue-resident macrophages function as sentinel cells that sense the presence of microbes and respond by secreting cytokines that initiate and then amplify the protective response against the microbes. Some of these cytokines act on endothelial cells lining blood vessels to enhance the recruitment of monocytes and other leukocytes from the blood into sites of infections. Other cytokines made by activated macrophages act on leukocytes and stimulate their migration to tissue sites of infection or damage. Some important macrophage-derived cytokines are discussed in Chapter 4.
- Macrophages that have engulfed microbes can be induced by microbial molecules to undergo an inflammatory form of death called pyroptosis, which usually results from activation of a cytoplasmic enzyme complex called the inflammasome, which is discussed in Chapter 4. Pyroptosis leads to the release of cytokines that enhance the host's inflammatory response to the infection.
- In addition to ingesting microbes, macrophages ingest necrotic host cells, including cells that die in tissues because of the effects of toxins, trauma or interrupted blood supply, and neutrophils that die after accumulating at sites of infection. This is part of the cleaning-up process after infection or sterile tissue injury. Macrophages can specifically recognize and engulf cells that die by apoptosis before the dead cells can release their contents and induce inflammatory responses. This clearance by macrophages of apoptotic cells, including apoptotic neutrophils, is a process called efferocytosis. Throughout the body and throughout the life of an individual, unwanted cells die by apoptosis as part of many physiologic processes, such as development and renewal of healthy tissues and maintenance of cell numbers (tissue homeostasis), and the dead cells are eliminated by macrophages.
- Macrophages serve as antigen-presenting cells (APCs) that display fragments of protein antigens to T lymphocytes and

activate T cells recruited to sites of injury or infection. This function is important in the effector phase of T cell–mediated immune responses (see Chapters 6 and 10).

- Macrophages promote the repair of damaged tissues by stimulating new blood vessel growth (angiogenesis) and synthesis of collagen-rich extracellular matrix (fibrosis). These functions are mediated by cytokines secreted by the macrophages that act on various tissue cells.

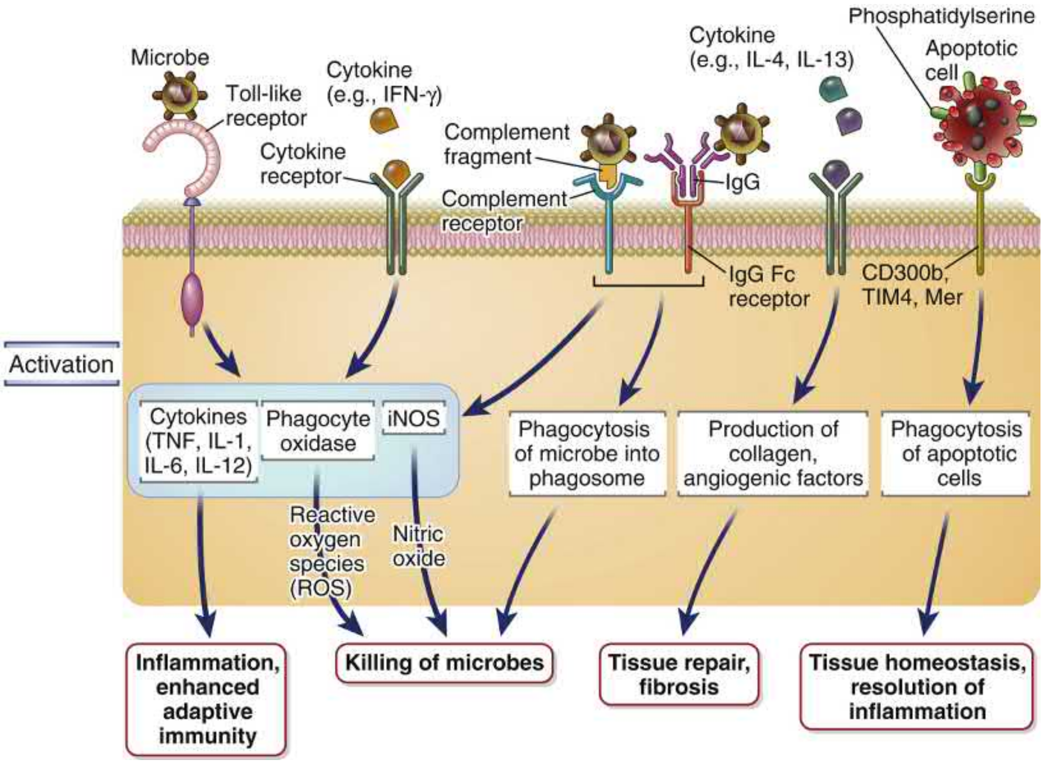

Microbe
Toll-like receptor
Cytokine (e.g., IFN-γ)
Cytokine receptor
Complement fragment
Complement receptor
IgG
IgG Fc receptor
Cytokine (e.g., IL-4, IL-13)
Phosphatidylserine
Apoptotic cell
CD300b, TIM4, Mer
Activation
Cytokines (TNF, IL-1, IL-6, IL-12)
Phagocyte oxidase
iNOS
Reactive oxygen species (ROS)
Nitric oxide
Phagocytosis of microbe into phagosome
Production of collagen, angiogenic factors
Phagocytosis of apoptotic cells
Inflammation, enhanced adaptive immunity
Killing of microbes
Tissue repair, fibrosis
Tissue homeostasis, resolution of inflammation

FIGURE 2.4 Functions of macrophages.Macrophages are activated by microbial products such as lipopolysaccharide and by natural killer cell–derived interferon-γ *(IFN-γ).* The process of macrophage activation leads to the activation of transcription factors, the transcription of various genes, and the synthesis of proteins that mediate the functions of these cells. In adaptive cell-mediated immunity, macrophages are activated by stimuli from T lymphocytes (CD40 ligand and IFN-γ) and respond in essentially the same way (see Fig. 10.7). Macrophages also may be activated by other signals to promote tissue repair and fibrosis (not shown). *IgG,* Immunoglobulin G; *IL,* interleukin; *iNOS,* inducible nitric oxide synthase; *TIM4,* T cell immunoglobulin 4.

Monocyte-derived macrophages may respond to microbes nearly as rapidly as neutrophils do, but macrophages survive much longer at sites of inflammation. Unlike neutrophils, macrophages can undergo cell division at an inflammatory site. Therefore, macrophages are the dominant effector cells of the later stages in the innate immune response, several days after an infection begins.

Macrophage Receptors and Activation

Macrophages are activated to perform their functions by recognizing many different kinds of microbial molecules, as well as host molecules produced in response to infections and injury. These various activating molecules bind to specific signaling receptors located on the surface of the macrophage (see Fig. 2.4). Examples of these receptors are the Toll-like receptors (TLRs), which are important in innate immunity and will be discussed in detail in Chapter 4. Macrophages are also activated when other plasma membrane receptors bind opsonins on the surface of microbes. Opsonins are substances that coat microbial cells or other particles and tag them for phagocytosis. Examples of opsonin receptors are complement receptors, which bind fragments of complement proteins attached to microbial surfaces, and immunoglobulin G (IgG) Fc receptors, which bind to one end of IgG antibody molecules that already have microbes bound at the other end, discussed in Chapter 13. Macrophage phagocytosis of healthy host cells is prevented in part by an inhibitory receptor on the macrophage called SIRPα, which recognizes CD47, a membrane protein on healthy cells that functions as a “don’t eat me” signal. When CD47 binds to SIRPα, inhibitory signals are generated in the macrophage that prevent phagocytosis. In adaptive immunity, macrophage antimicrobial functions are activated by some T lymphocyte cytokines and membrane proteins that bind to signaling receptors on the macrophage membrane (see Chapter 10).

Subsets of Macrophages

Macrophages can acquire distinct functional capabilities, depending on the types of activating stimuli to which they are exposed. The clearest example of this is the response of macrophages to different cytokines made by subsets of T cells. Some of these cytokines activate macrophages to become efficient at killing microbes, called **classical activation,** and these cells are called M1 macrophages**.** Other cytokines activate macrophages to promote tissue remodeling and repair, called **alternative activation,** and these cells are called M2 macrophages. These different pathways of activation and the cytokines involved are discussed in Chapter 10. The relationship between blood monocyte subsets, discussed earlier, and macrophage subsets is not well understood. Macrophages may also assume different morphologic forms after activation by external stimuli, such as microbes. Some develop abundant cytoplasm and are called epithelioid cells because of their resemblance to epithelial cells of the skin. Activated macrophages can fuse to form multinucleated giant cells, which occurs frequently in certain types of microbial infections, such as with mycobacteria, and in response to indigestible foreign bodies.

Mast Cells, Basophils, and Eosinophils

Mast cells, basophils, and eosinophils are three additional cell types that play roles in innate and adaptive immune responses. All three share the common property of having cytoplasmic granules filled with various inflammatory and antimicrobial mediators, which are released from the cells upon activation. Another common feature of these cells is their involvement in immune responses that protect against helminths and reactions that cause allergic diseases. We will introduce the features of these cells in this section and discuss their functions in more detail in Chapter 20.

Mast Cells

Mast cells are bone marrow–derived cells that are most abundant in the skin and mucosal epithelia; upon activation, they release many potent inflammatory mediators that defend against infections by

helminthic parasites or cause symptoms of allergic diseases. A cytokine called stem cell factor (or c-KIT ligand) is essential for mast cell development. Normally, mature mast cells are not found in the circulation but are present in tissues, usually adjacent to small blood vessels and nerves (Fig. 2.1B). Their cytoplasm contains numerous membrane-bound granules, which are filled with preformed inflammatory mediators, such as histamine, and acidic proteoglycans that bind basic dyes, imparting a dark blue color to the granules when special stains are used. Various stimuli can activate mast cells to release the cytoplasmic granule contents into the extracellular space, as well as to synthesize and release cytokines and inflammatory lipid mediators. The released histamine and other mediators promote changes in the blood vessels that cause inflammation. Mast cells express high-affinity plasma membrane receptors for a type of antibody called IgE and are usually coated with these antibodies. When the antibodies on the mast cell surface bind antigen, signaling events are induced that lead to mast cell activation. Mast cells are also activated when they recognize microbial products, independent of IgE, and in this way they function as tissue sentinels of the innate immune system.

Basophils

Basophils are blood granulocytes with many structural and functional similarities to mast cells. Like other granulocytes, basophils are derived from hematopoietic precursors, mature in the bone marrow (from progenitors distinct from those of mast cells), and circulate in the blood. Basophils constitute less than 1% of blood leukocytes (see Table 2.1). Although they are normally not present in tissues, basophils may be recruited to some inflammatory sites. These cells also contain granules that bind basic dyes (Fig. 2.1C), and they are capable of synthesizing many of the same mediators as mast cells. Like mast cells, basophils express IgE receptors, bind IgE, and can be triggered by antigen binding to IgE. Because basophil numbers are low in tissues, their importance in host defense and allergic reactions is uncertain.

Eosinophils

Eosinophils are granulocytes that express cytoplasmic granules containing enzymes that are harmful to the cell walls of parasites but also can damage host tissues. Eosinophil granules contain mainly basic proteins that bind acidic dyes, such as eosin, and thus appear red in stained blood smears and tissue sections (Fig. 2.1D). Eosinophils are bone marrow–derived and circulate in the blood, from where they may be recruited into tissues. The cytokines GM-CSF, interleukin-3 (IL-3), and IL-5 promote eosinophil maturation from myeloid precursors. Various membrane receptors on eosinophils, including Fc receptors for IgA and IgG, TLRs, and IL-5 receptors, can generate signals that activate the cells to release their granule contents Some eosinophils are normally present in peripheral tissues, especially in mucosal linings of the respiratory, gastrointestinal, and genitourinary tracts, and their numbers can increase by recruitment from the blood in the setting of inflammation.

Dendritic Cells (DCs)

DCs are tissue-resident and circulating cells that detect the presence of microbes and initiate innate immune defense reactions, and they capture microbial proteins for display to T cells to initiate adaptive immune responses. These cells are named because of their long membranous projections, reminiscent of the dendrites of neurons. Most DCs are widely distributed in lymphoid tissues, mucosal epithelium, and organ parenchyma (Fig. 2.5). The location of DCs in epithelia and tissues where microbes enter, their ability to capture antigens and take them to lymph nodes where naive T cells circulate, and their rapid responses to microbes all place these cells in a unique position in the immune system, serving as sentinels of infection that begin the rapid innate response but also link innate responses with the development of adaptive immune responses. We will discuss the role of DCs as mediators of innate immunity and as APCs in Chapters 4 and 6, respectively. Here we will introduce the general properties of DCs.

Development and Features of Dendritic Cell Subsets

Subsets of DCs can be defined on the basis of different cell surface markers, transcription factors, development from different precursor cells, tissue localization, and functions. We will describe the major subsets that are important in immune responses and are distinguished from one another by their functions and development, and by expression of different surface molecules and transcription factors (Fig. 2.6 and Table 2.3). The common properties of these DC subsets include developmental dependence on the cytokine FLT3L, expression of the CD11c protein, and the ability to present antigens to and activate naive T cells or induce T cell tolerance.

- ***Classical DCs (also called conventional DCs [cDCs]) are the major type of DC involved in capturing protein antigens of microbes that enter through epithelial barriers and presenting them to T cells.*** Classical DCs were first identified by their morphology and ability to stimulate strong T cell responses and are the most numerous DC subset in epithelia and lymphoid organs. They arise from bone marrow HSCs through a developmental pathway that includes a common precursor of both monocytes and classical DCs, some of which develop into committed precursors for cDCs (called pre-cDCs). All of these steps take place in the bone marrow. The pre-cDCs migrate to peripheral tissues, where they mature into cDCs. Similar to tissue macrophages, these DCs constantly sample the environment in which they reside.

Classical DCs may be further divided into two main subsets called major, or cDC2, and cross-presenting, or cDC1 (see Fig. 2.6 and Table 2.3). cDC2 is the most numerous subset and is potent at capturing exogenous antigens and inducing CD4$^+$ T cell responses. The cDC1 subset is specialized to present antigens to naive CD8$^+$ T cells by a process called cross-presentation, discussed in Chapter 6; this subset can also present antigens to CD4$^+$ cells.

- ***Plasmacytoid DCs (pDCs) produce the antiviral cytokine type I interferon (IFN) in response to viruses and may capture blood borne microbes and carry their antigens to the spleen for presentation to T cells.*** These DCs are named because after activation, they begin to resemble plasma cells morphologically. They develop in the bone marrow from a precursor distinct from that for classical DCs and are found in the blood and in small numbers in lymphoid organs. Plasmacytoid DCs are the body's major producers of cytokines called type I IFNs, which have potent antiviral activities and play an important role in innate host defense against viruses (see Chapter 4).
- ***Monocyte-derived DCs (MoDCs) include cells with functions similar to those of cDCs but are derived from monocytes that were recruited into tissue inflammatory sites.*** They express CD11c, like all DCs, and also monocyte markers such as CD11b and CCR2.
- ***Langerhans cells are DCs found in the epidermis that share functions with cDCs but are developmentally related to tissue-resident macrophages, arising from embryonic fetal liver and yolk sac precursors*** (see Fig. 2.6) **.** They are identified by their location and morphology in the skin, the presence of tennis-racket– shaped cytoplasmic organelles called Birbeck granules that are visible using electron microscopy, and expression of various markers (see Table 2.3). Langerhans cells may function in the context of skin infections to present antigens to and activate CD4$^+$ T cells, or in the absence of infection, to present self antigens to CD4$^+$ T cells and induce tolerance to these antigens.

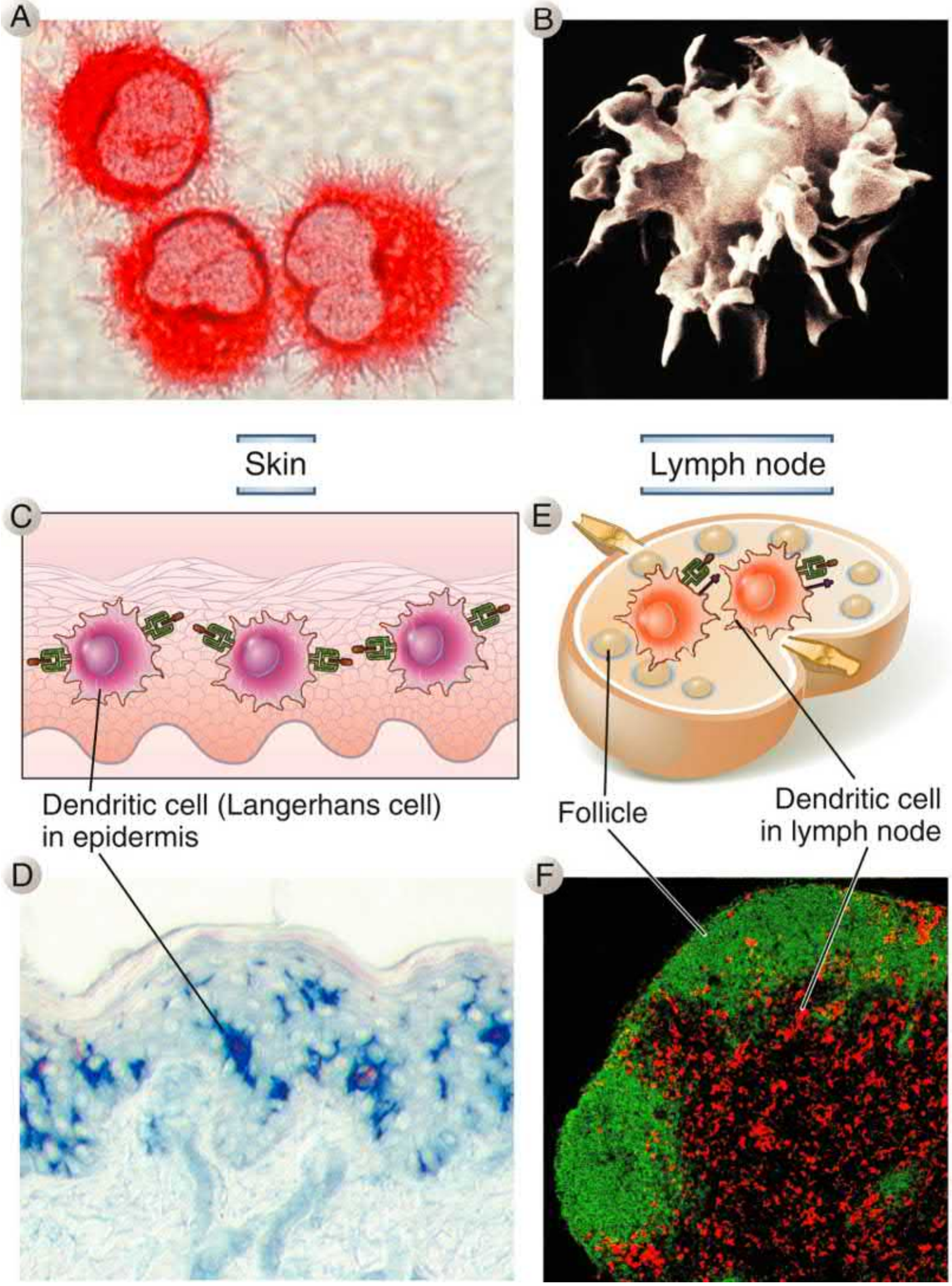

A
B
Skin
Lymph node
C
E
Dendritic cell (Langerhans cell) in epidermis
Follicle
Dendritic cell in lymph node
D
F

FIGURE 2.5 Dendritic cells. **A,** Light micrograph of cultured dendritic cells (DCs) derived from bone marrow precursors. **B,** A scanning electron micrograph of a DC showing extensive membrane projections. **C** and **D,** DCs in the skin, illustrated schematically **(C)** and in a section of the skin **(D)**

stained with an antibody specific for Langerhans cells (which appear *blue* in this immunoenzyme stain). **E** and **F,** DCs in a lymph node, illustrated schematically **(E)** and in a section of a mouse lymph node **(F)** stained with fluorescently labeled antibodies against B cells in follicles *(green)* and DCs in the T cell zone *(red).*

A, B, and *D,* Courtesy of Dr. Y-J Liu, MD, Anderson Cancer Center, Houston, Texas. *F,* Courtesy of Drs. Kathryn Pape and Jennifer Walter, University of Minnesota School of Medicine, Minneapolis, Minnesota

Another population of cells called **follicular dendritic cells** (FDCs) have a dendritic morphology but are not derived from bone marrow precursors, do not present protein antigens to T cells, and should not be confused with DCs. FDCs are involved in B cell activation in the germinal centers of secondary lymphoid organs (see Chapter 12).

Lymphocytes

Lymphocytes, the unique cells of adaptive immunity, are the only cells in the body that express clonally distributed antigen receptors, each specific for a different antigenic determinant. Each clone of T and B lymphocytes expresses antigen receptors with a single specificity, which is different from the specificities of the receptors in all other clones. As we shall discuss here and in later chapters, there are millions of lymphocyte clones in the body, enabling any individual to recognize and respond to millions of foreign antigens.

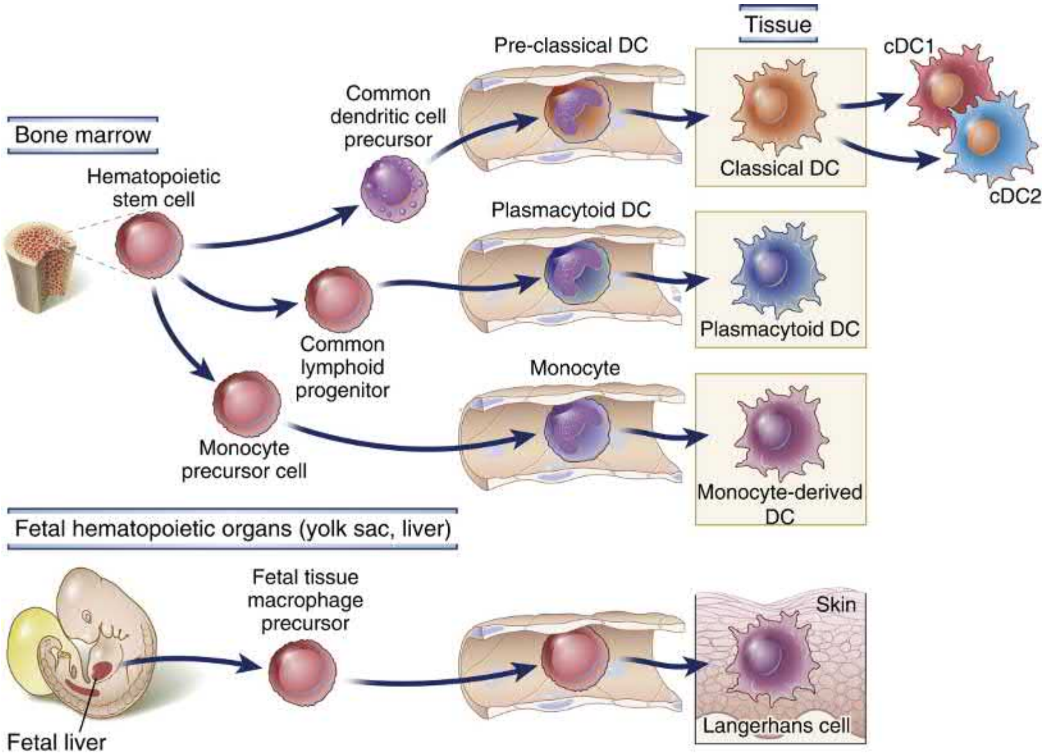

Tissue
Pre-classical DC
cDC1
Common
dendritic cell
precursor
Bone marrow
Hematopoietic
stem cell
Classical DC
cDC2
Plasmacytoid DC
Plasmacytoid DC
Common
lymphoid
progenitor
Monocyte
Monocyte
precursor cell
Monocyte-derived
DC
Fetal hematopoietic organs (yolk sac, liver)
Fetal tissue
macrophage
precursor
Skin
Fetal liver
Langerhans cell

FIGURE 2.6 Maturation of dendritic cells.Dendritic cells *(DCs)* arise from a common precursor cell of the myeloid lineage in the bone marrow and further differentiate into subsets, the major ones being classical DCs *(cDCs)* and plasmacytoid DCs *(pDCs)*. Monocyte-derived DCs *(Mo-DCs)* may arise from monocytes in inflamed tissues Some plasmacytoid DCs may arise from the common DC precursor.

The role of lymphocytes in mediating adaptive immunity was established by several lines of evidence accumulated over decades of research. One of the earliest clues came from the observation that humans with congenital and acquired immunodeficiency states had reduced numbers of lymphocytes in the peripheral circulation and in lymphoid tissues. Experiments done in mice and rats showed that depletion of lymphocytes impaired responses to immunizations, and lymphocytes are the only cell type that can transfer specific immunity to microbes from immunized to naive animals. In vitro experiments established that stimulation of lymphocytes with antigens leads to responses that show many of the characteristics of immune responses induced under more physiologic conditions in vivo. After the identification of lymphocytes as the mediators of humoral and cellular immunity, many discoveries were made at a rapid pace about different types of lymphocytes, their origins in the bone marrow and thymus, their roles in various immune responses, and the consequences of their deficiency. Among the most important findings was that clonally distributed, highly diverse, and specific receptors for antigens are produced by lymphocytes but not by any other types of cells. More recently, an enormous amount of information has accumulated about lymphocyte genes, proteins, and functions.

One of the most fascinating aspects of lymphocyte biology is how the extremely diverse repertoire of antigen receptors with different specificities is generated from the limited number of genes for these receptors that are present in the germline. It is now known that the genes encoding the antigen receptors of lymphocytes are formed by recombination of DNA segments during the maturation of these cells. There is a random aspect to these somatic recombination events that results in the generation of millions of different recombined receptor genes and a highly diverse repertoire of antigen specificities among different clones of lymphocytes (see Chapter 8).

The total number of lymphocytes in a healthy adult is approximately 5×10^{11}. Of these, approximately 2% are in the blood, 4% in the skin, 10% in the bone marrow, 15% in the mucosal lymphoid tissues of the gastrointestinal and respiratory tracts, and 65% in lymphoid organs (mainly the spleen and lymph nodes). We first describe the properties of these cells and then their organization in various lymphoid tissues.

TABLE 2.3

	Classical (Conventional) Dendritic Cells				
	cDC2	**cDC1 (Cross-Presenting)**	**Plasmacytoid Dendritic Cells**	**Langerhans Cells**	**Monocyte-Derived DCs**
Surface markers	CD11c BDCA-1 (CD1c)	CD11c BDCA-3 (CD141) CLEC9A XCR1$^+$	BDCA-2 (CD303) BDCA-4 (CD304) CD123	CD11b Langerin (CD207) EPCAM BDCA1 CD1a	CD11b CCR2 CD14
TLRs expressed	Various	Various	High levels of TLR7, TLR9	Various	Various
Transcription factors	IRF4	IRF8, BATF3	E2-2	PU.1	
Major cytokines produced	Various (including IL-6, IL-23)	IL-12	Type I IFN		Various
Major postulated functions	Innate immunity: source of inflammatory cytokines Adaptive immunity: capture and presentation of antigens mostly to CD4$^+$ T cells	Adaptive immunity: capture and cross-presentation of antigens to CD8$^+$ T cells; induction of Th1 responses	Antiviral immunity: early innate response; priming of antiviral T cells	Innate immunity: source of inflammatory cytokines Adaptive immunity: capture and presentation of antigens mostly to CD4$^+$ T cells	Innate immunity: source of inflammatory cytokines Role in adaptive immunity unclear

The major distinguishing markers and functions of human dendritic cell (DC) subsets are listed. Note that all DCs express class II major histocompatibility complex molecules. Unactivated DCs of all types may display self antigens and serve to maintain self-tolerance; this postulated function is not listed in the table.

IL, Interleukin; *IFN,* interferon; *TLRs,* Toll-like receptors.

Classes of Lymphocytes

Lymphocytes consist of distinct classes with different functions and protein products (Table 2.4) **.** The major classes of lymphocytes were introduced in Chapter 1 (see Fig. 1.5). Morphologically, all lymphocytes are similar, and their appearance does not reflect their heterogeneity or their diverse functions. **B lymphocytes,** the cells that produce antibodies, were so called because in birds they were found to mature in an organ called the bursa of Fabricius. In mammals, no anatomic equivalent of the bursa exists, and the early stages of B cell maturation occur in the bone marrow. Thus, the name B lymphocytes now refers to bone marrow–derived lymphocytes. **T lymphocytes,** the mediators of cellular immunity, arise from precursor cells in the bone marrow, which migrate to and mature in the thymus; T lymphocytes refer to thymus-derived lymphocytes. There are subsets of B and T lymphocytes with distinct phenotypic and functional characteristics.

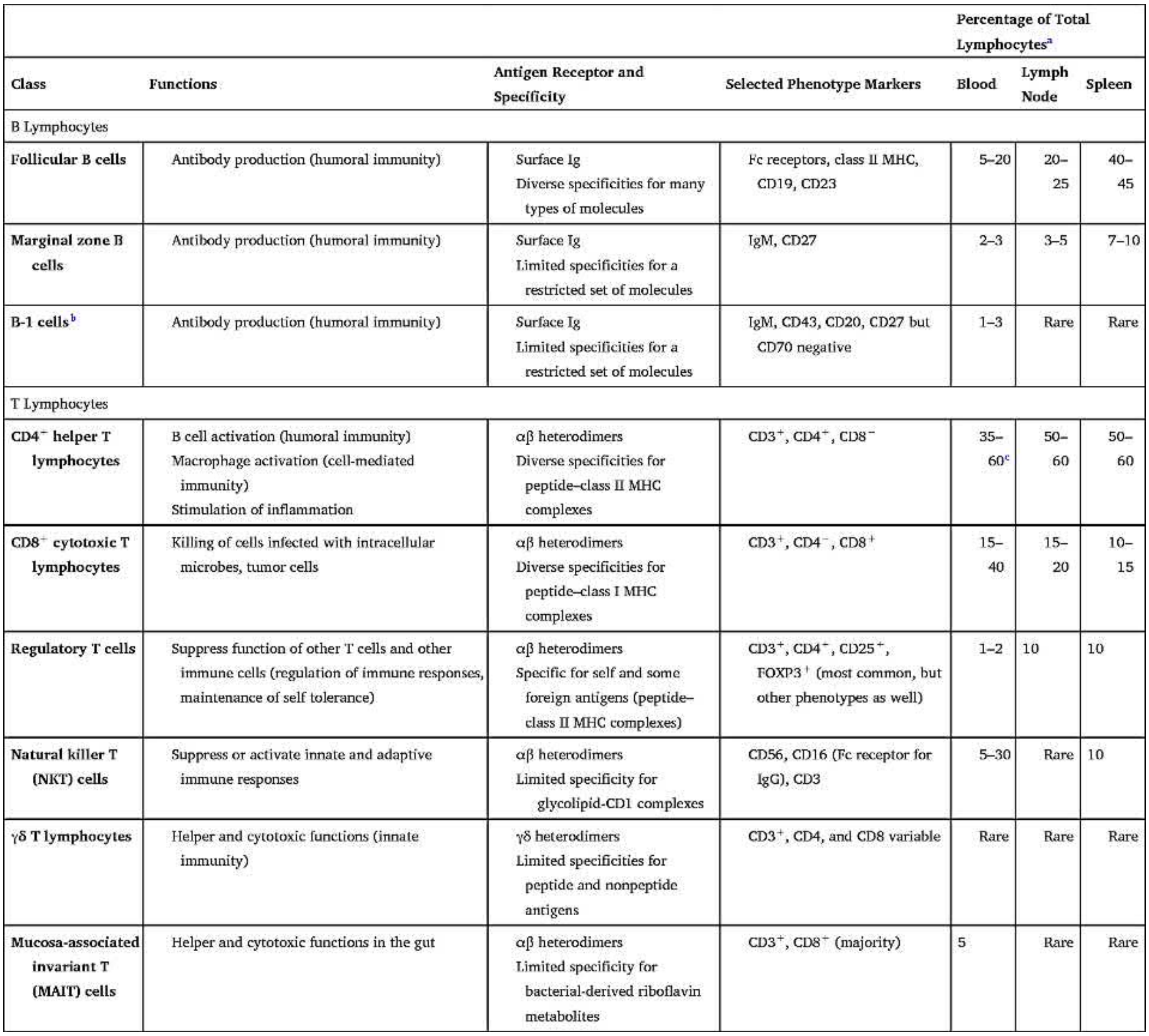
Subsets of B Lymphocytes

The major subsets of B cells are follicular B cells, marginal zone B cells, and B-1 cells, each of which is found in distinct anatomic locations within lymphoid tissues. Follicular B cells, the most numerous type of B cells in the body, are found in lymphoid tissues and blood. They express highly diverse, clonally distributed sets of antibodies that serve as cell surface antigen receptors and as the key secreted effector molecules of adaptive humoral immunity. Follicular B cells give rise to most of the high-affinity antibodies and memory B cells that protect people from repeat infections by the same microbes. In contrast, B-1 and marginal zone B cells make up a minority of B cells and produce antibodies with limited diversity. B-1 cells are found mainly in mucosal tissues and the peritoneal and pleural cavities, whereas marginal zone B cells are present only in the spleen in rodents but can be found in the circulation of humans.

Subsets of T Lymphocytes

The two major T cell subsets are defined by the cell surface expression of the CD4 and CD8 proteins (Fig. 2.7). T cells are the mediators of cellular immunity: $CD4^+$ T cells are helper T lymphocytes or their naive precursors, and $CD8^+$ T cells are CTLs or their precursors. Both $CD4^+$ and $CD8^+$ T cells express antigen receptors called $\alpha\beta$ T cell receptors (TCRs). $CD4^+$ helper T cells secrete cytokines that act on various other cells, including other T lymphocytes, B cells, and macrophages. $CD8^+$ CTLs recognize and kill cells infected with viruses and other microbes that can live inside host cells, and also kill cancer cells. $CD4^+$ regulatory T cells are a third subset of T cells expressing $\alpha\beta$ receptors; their function is to inhibit immune responses. In addition, natural killer T (NKT) cells, mucosa-associated invariant T (MAIT) cells, and $\gamma\delta$ T cells are three numerically smaller subsets of T cells that express TCRs with limited diversity, analogous to the antibodies made by B-1 cells. The functions of these classes of B and T cells will be discussed in later chapters.

TABLE 2.4



				Percentage of Total Lymphocytes[a]		
Class	**Functions**	**Antigen Receptor and Specificity**	**Selected Phenotype Markers**	**Blood**	**Lymph Node**	**Spleen**
B Lymphocytes						
Follicular B cells	Antibody production (humoral immunity)	Surface Ig Diverse specificities for many types of molecules	Fc receptors, class II MHC, CD19, CD23	5–20	20–25	40–45
Marginal zone B cells	Antibody production (humoral immunity)	Surface Ig Limited specificities for a restricted set of molecules	IgM, CD27	2–3	3–5	7–10
B-1 cells[b]	Antibody production (humoral immunity)	Surface Ig Limited specificities for a restricted set of molecules	IgM, CD43, CD20, CD27 but CD70 negative	1–3	Rare	Rare
T Lymphocytes						
$CD4^+$ helper T lymphocytes	B cell activation (humoral immunity) Macrophage activation (cell-mediated immunity) Stimulation of inflammation	αβ heterodimers Diverse specificities for peptide–class II MHC complexes	$CD3^+$, $CD4^+$, $CD8^-$	35–60[c]	50–60	50–60
$CD8^+$ cytotoxic T lymphocytes	Killing of cells infected with intracellular microbes, tumor cells	αβ heterodimers Diverse specificities for peptide–class I MHC complexes	$CD3^+$, $CD4^-$, $CD8^+$	15–40	15–20	10–15
Regulatory T cells	Suppress function of other T cells and other immune cells (regulation of immune responses, maintenance of self tolerance)	αβ heterodimers Specific for self and some foreign antigens (peptide–class II MHC complexes)	$CD3^+$, $CD4^+$, $CD25^+$, $FOXP3^+$ (most common, but other phenotypes as well)	1–2	10	10
Natural killer T (NKT) cells	Suppress or activate innate and adaptive immune responses	αβ heterodimers Limited specificity for glycolipid-CD1 complexes	CD56, CD16 (Fc receptor for IgG), CD3	5–30	Rare	10
γδ T lymphocytes	Helper and cytotoxic functions (innate immunity)	γδ heterodimers Limited specificities for peptide and nonpeptide antigens	$CD3^+$, CD4, and CD8 variable	Rare	Rare	Rare
Mucosa-associated invariant T (MAIT) cells	Helper and cytotoxic functions in the gut	αβ heterodimers Limited specificity for bacterial-derived riboflavin metabolites	$CD3^+$, $CD8^+$ (majority)	5	Rare	Rare

This table summarizes the major properties of the lymphocytes of the adaptive immune system. Not included are natural killer cells and other innate lymphoid cells, which are discussed in Chapter 4.

Ig, Immunoglobulin; *MHC,* major histocompatibility complex.

[a] The percentages are approximations, based on data from human peripheral blood and mouse lymphoid organs. In the liver, almost 50% of the lymphocytes are MAIT cells.

[b] B-1 cells are a distinct subset in mice, but it is unclear if the same is true in humans.

[c] In most cases the ratio of $CD4^+CD8^-$ to $CD8^+CD4^-$ is approximately 2:1 in steady state.

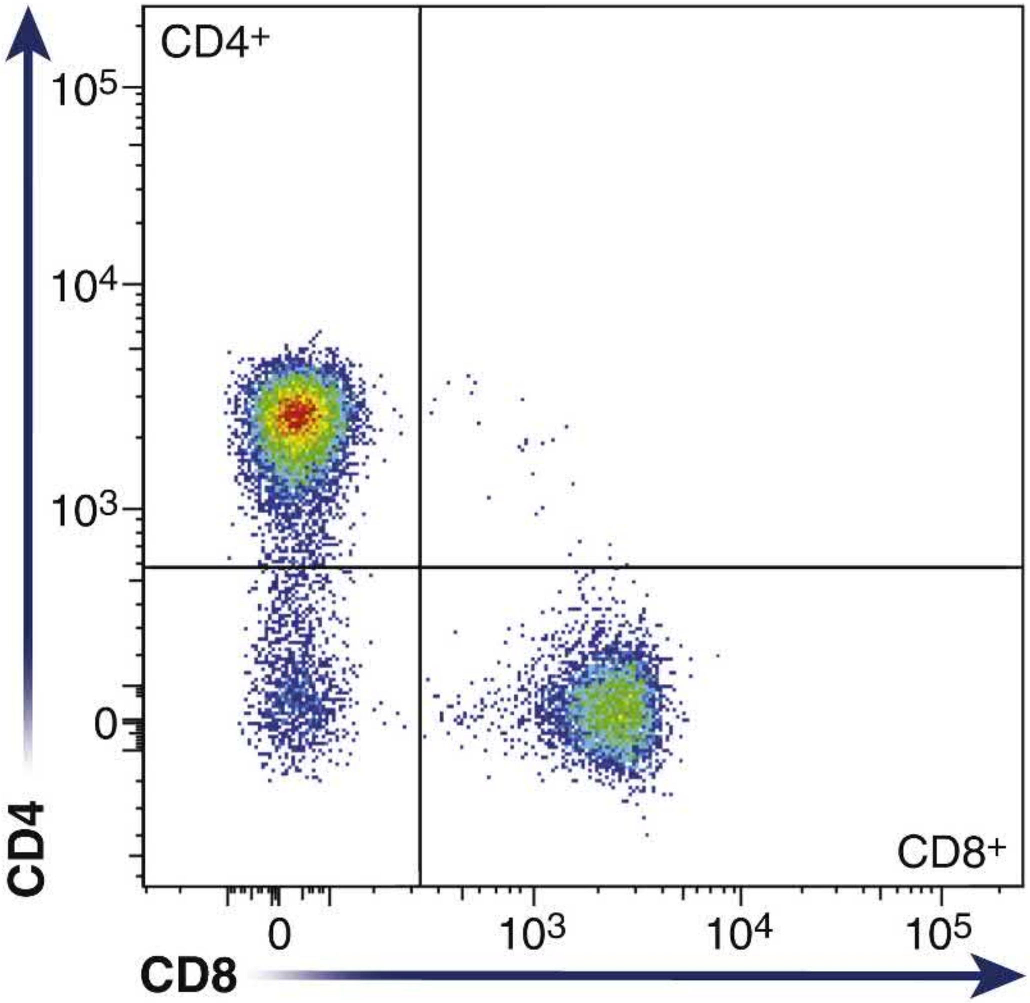


FIGURE 2.7 T cell subsets.A flow cytometry plot is shown of human blood lymphocytes that have been stained with reagents to detect the cell surface expression of CD4 or CD8. Each dot represents a single cell, and the location of the dot within the plot reflects that cell's expression of CD4 and CD8. (Flow cytometry is explained in Appendix III). Most T cells in blood and lymphoid organs express CD4 or CD8 but not both, and in healthy people, the ratio of $CD4^+$ to $CD8^+$ T cells is about 2:1.

Courtesy of Mariela Pauli and Robby Grewal, Department of

Dermatology, University of California San Francisco, San Francisco.

Development of Lymphocytes

Lymphocytes, like all blood cells, arise after birth from stem cells in the bone marrow. The origin of lymphocytes from bone marrow progenitors was first demonstrated by experiments with radiation-induced bone marrow chimeras. Lymphocytes and their precursors are radiosensitive and are killed by high doses of γ-irradiation. If a mouse of one inbred strain is irradiated and then injected with bone marrow cells or small numbers of HSCs of another strain that can be distinguished from the host, all the lymphocytes that develop subsequently are derived from the bone marrow cells or HSCs of the donor. Such approaches have proved useful for examining the maturation of lymphocytes and other blood cells.

All lymphocytes go through complex maturation stages during which they express antigen receptors and acquire the functional and phenotypic characteristics of mature cells (Fig. 2.8). The anatomic sites where the major steps in lymphocyte development occur are called the generative (or primary, or central) lymphoid organs. These include the bone marrow, where precursors of all lymphocytes arise and B cells mature, and the thymus, where T cells mature. We will discuss the processes of B- and T-lymphocyte maturation in detail in Chapter 8.

Populations of Lymphocytes Distinguished by History of Antigen Exposure

Naive lymphocytes that have matured in the bone marrow or thymus migrate into secondary (peripheral) lymphoid organs, where they are activated by antigens to proliferate and differentiate into effector and memory cells (Fig. 2.9 and Table 2.5) **.** The mature T cells that emerge from the thymus are called **naive T lymphocytes.** B cells undergo most of their development in the bone marrow, but the final steps that generate mature **naive B lymphocytes** occur in the spleen. Naive lymphocytes are functionally quiescent, but after activation by antigen, they proliferate and go through dramatic changes in phenotype and functional activity. The activation of naive lymphocytes follows a series of sequential steps beginning with the synthesis of new proteins, such as cytokine receptors and cytokines, which are required for many of the subsequent changes. The cells then undergo proliferation, resulting in increased size of the antigen-specific clones, a process called **clonal expansion.** In some infections the number of microbe-specific T cells may increase more than 50,000-fold within a week, and the number of specific B cells may increase up to 5000-fold. This rapid clonal expansion of microbe-specific lymphocytes is needed to keep pace with the ability of microbes to rapidly replicate. Concurrently with proliferation, antigen-stimulated lymphocytes begin to differentiate into **effector cells** whose function is to eliminate the antigen. Many of the effector cells migrate into tissue sites of infection, and some stay in secondary lymphoid organs. Other progeny of antigen-stimulated B and T lymphocytes differentiate into long-lived **memory cells,** whose function is to mediate rapid and enhanced (i.e., secondary) responses to subsequent exposures to antigens. Naive, effector, and memory lymphocytes can be distinguished by several functional and phenotypic criteria (see Table 2.5).

The details of lymphocyte activation and differentiation, as well as the functions of each of these populations, will be addressed later in the book. Here we will summarize the phenotypic characteristics of each population.

Naive Lymphocytes

Naive lymphocytes are mature T or B cells that have never encountered foreign antigen. (The term naive refers to the idea that these cells are immunologically inexperienced.) Naive lymphocytes are found in the circulation and secondary lymphoid organs. Naive and memory lymphocytes are both called resting lymphocytes because they are not actively dividing or performing effector functions and are in a state of rest, or in the G0 stage of the cell cycle, before antigenic stimulation. Naive (and memory) B and T lymphocytes cannot be readily distinguished morphologically, and both are often called small lymphocytes when observed in blood smears. A small lymphocyte is 8 to 10 μm in diameter and has a large nucleus with dense heterochromatin and a thin rim of cytoplasm that contains a few mitochondria, ribosomes, and lysosomes but no visible specialized organelles (Fig. 2.10). Naive (and memory) lymphocytes rely mainly on oxidative phosphorylation and fatty acid oxidation to maintain their basal energy requirements.

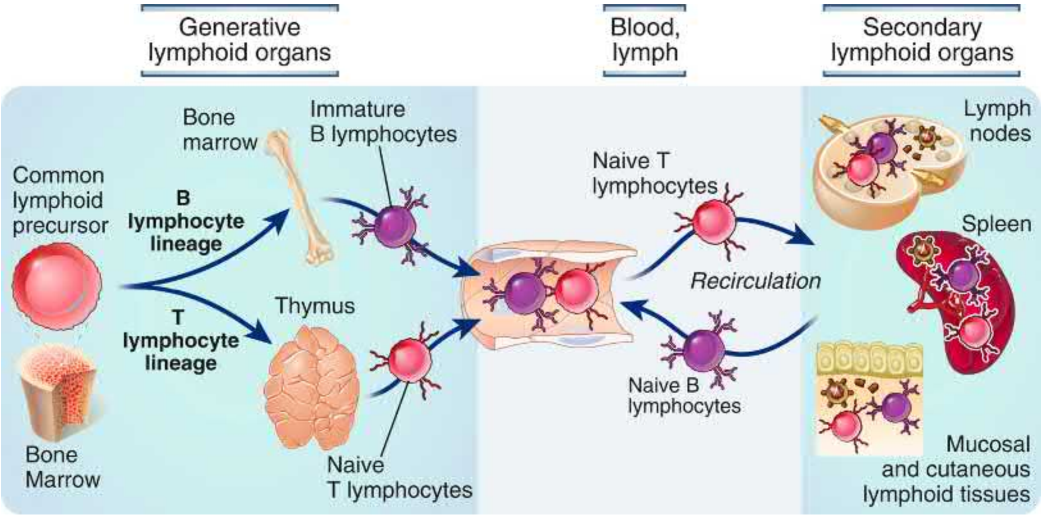

Generative lymphoid organs
Blood, lymph
Secondary lymphoid organs
Common lymphoid precursor
Bone marrow
Immature B lymphocytes
B lymphocyte lineage
T lymphocyte lineage
Thymus
Bone Marrow
Naive T lymphocytes
Naive T lymphocytes
Recirculation
Naive B lymphocytes
Lymph nodes
Spleen
Mucosal and cutaneous lymphoid tissues

FIGURE 2.8 Maturation of lymphocytes.Lymphocytes develop from bone marrow stem cells, mature in the generative lymphoid organs (bone marrow and thymus for B and T cells, respectively), and then circulate through the blood to secondary lymphoid organs (lymph nodes, spleen, mucosal lymphoid tissues). Fully mature T cells leave the thymus, but immature B cells leave the bone marrow and complete their maturation in secondary lymphoid organs. Naive lymphocytes may respond to foreign antigens in these secondary lymphoid tissues or return by lymphatic drainage to the blood and recirculate through other secondary lymphoid organs.

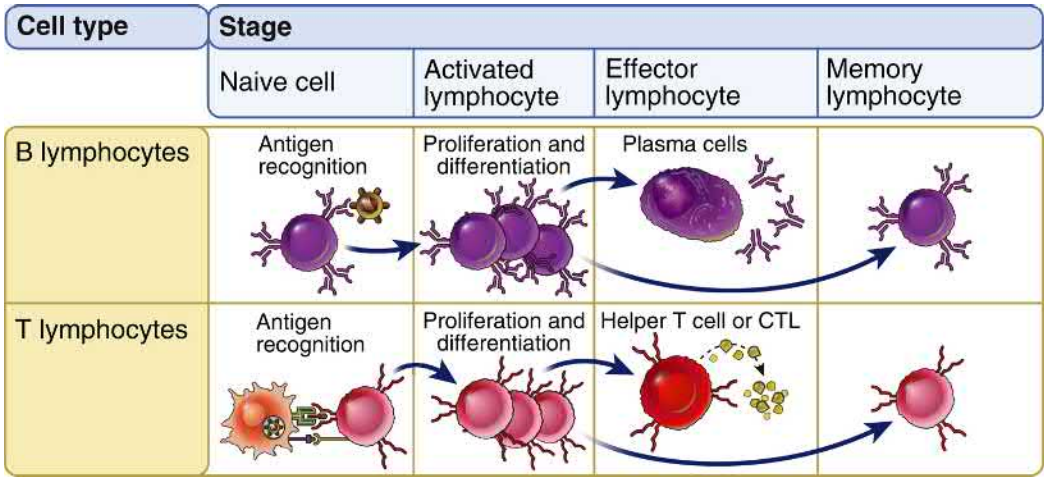

Cell type
Stage
Naive cell
Activated lymphocyte
Effector lymphocyte
Memory lymphocyte
B lymphocytes
Antigen recognition
Proliferation and differentiation
Plasma cells
T lymphocytes
Antigen recognition
Proliferation and differentiation
Helper T cell or CTL

FIGURE 2.9 Stages in the life history of lymphocytes.In response to antigen, naive lymphocytes in secondary lymphoid proliferate and differentiate into effector cells, which function to eliminate antigens. The effector cells of the B lymphocyte lineage are antibody-secreting plasma cells (some of which are long-lived). The effector cells of the CD4 T lymphocyte lineage are cytokine-producing helper T cells, and the effector cells of the CD8 lineage (not shown) are cytotoxic T lymphocytes. Most effector T cells leave secondary lymphoid organs and migrate into infected tissues. Some helper T cells remain in the secondary lymphoid organs, where they help B cells mount antibody responses. Other progeny of the antigen-stimulated lymphocytes differentiate into long-lived memory cells, which are located in secondary lymphoid organs and nonlymphoid tissues. See Table 2.5 for features of naive, effector, and memory lymphocytes.

TABLE 2.5

	Naive	Activated or Effector	Memory
T Lymphocytes			
Migration	Preferentially to secondary lymphoid organs	Preferentially to inflamed tissues	Preferentially to inflamed tissues, mucosal tissues
Frequency of cells responsive to particular antigen	Very low	High	Low
Effector functions	None	Cytokine secretion; cytotoxic activity	None
Cell cycling	No	Yes	±
Surface Protein Expression			
IL-2R (CD25)	Low	High	Low
L-selectin (CD62L)	High	Low	Variable
IL-7R (CD127)	Moderately high	Low	High
Adhesion molecules: integrins, CD44	Low	High	High
Chemokine receptor: CCR7	High	Low	Variable
Major CD45 isoform (humans only)	CD45RA	CD45RO	CD45RO; variable
Morphology	Small; scant cytoplasm	Large; more cytoplasm	Small
B Lymphocytes			
Ig produced	Membrane IgM and IgD	Frequently secreted IgG, IgA, IgE	Membrane IgG, IgA, IgE
Affinity of Ig	Relatively low	Increases during immune response	Relatively high
Effector function	None	Antibody secretion	None
Morphology	Small; scant cytoplasm	Large; more cytoplasm; plasma cell	Small
Surface Protein Expression			
Chemokine receptor: CXCR5	High	Low	?
CD27	Low	High	High

Ig, Immunoglobulin; *IL,* interleukin.

Naive lymphocytes typically live for 1 to 3 months. Their survival requires signals from antigen receptors and cytokines. The need for antigen receptor expression to maintain the pool of naive lymphocytes in secondary lymphoid organs was demonstrated in studies with mice in which the genes that encode the antigen receptors of B cells or T cells were deleted after the lymphocytes had matured. In these studies, naive lymphocytes that lost their antigen receptors died within 2 or 3 weeks. It has been shown that the antigen receptor of naive B cells generates survival signals even in the absence of antigen. Naive T lymphocytes recognize various self antigens weakly, enough to induce survival signals but without triggering the stronger signals that are needed to initiate proliferation and differentiation into effector cells.

Cytokines are also essential for the survival of naive lymphocytes, and naive B and T cells express receptors for these cytokines. The most important of these cytokines are IL-7, which promotes survival and low-level cycling of naive T cells, and B cell–activating factor (BAFF), a cytokine belonging to the tumor necrosis factor (TNF) family, which is required for naive B cell survival.

In the steady state, or homeostasis, the pool of naive lymphocytes is maintained at a fairly constant number because of a balance between spontaneous death of these cells and the production of new cells in the generative lymphoid organs. Any loss of lymphocytes leads to a compensatory proliferation of the remaining ones and increased output from the primary organs. This response of the immune system to reestablish a normal total number of lymphocytes is called homeostatic proliferation. If naive cells are transferred into a host that is deficient in lymphocytes (said to be lymphopenic), the transferred lymphocytes begin to proliferate and increase in number until they reach approximately the numbers of lymphocytes in normal animals. Homeostatic proliferation appears to be driven by the same signals that are required for the maintenance of naive lymphocytes, namely weak recognition of some self antigens in the case of T cells or spontaneous B cell receptor signaling in B cells, and cytokines, mainly IL-7. This phenomenon is exploited clinically in T cell therapy protocols, as in the treatment of some leukemias—the transferred T cells proliferate maximally if host T cell numbers are reduced, a process called lymphodepletion (see Chapter 18).

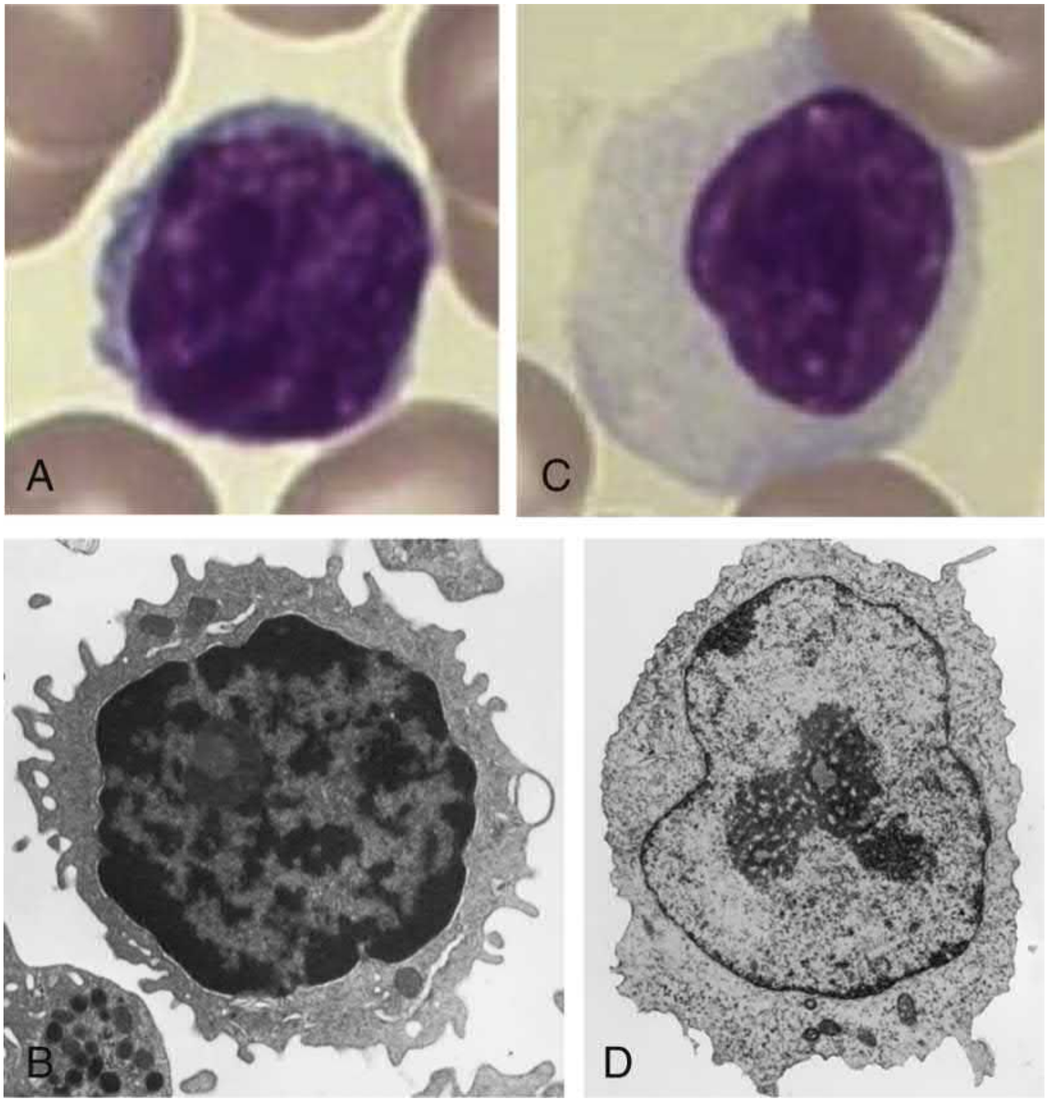


FIGURE 2.10 Morphology of lymphocytes. **A,** Light micrograph of a lymphocyte in a peripheral blood smear. **B,** Electron micrograph of a small lymphocyte. **C,** Light micrograph of a large lymphocyte (lymphoblast). **D,** Electron micrograph of a large lymphocyte (lymphoblast).

A, Courtesy of Jean Shafer, Department of Pathology, University of California, San Diego. Copyright 1995–2008, Carden Jennings Publishing Co., Ltd. *B,* Courtesy of Dr. Noel Weidner, Department of Pathology, University of California, San Diego. *C,* Courtesy of Jean Shafer, Department of Pathology, University of California, San Diego. Copyright 1995–2008, Carden Jennings Publishing Co., Ltd.

D, From Fawcett DW. *Bloom and Fawcett: A Textbook of Histology.* 12th ed. New York, NY: Chapman & Hall; 1994. With kind permission of Springer Science and Business Media.

Effector Lymphocytes

In response to stimulation by antigen and other signals, naive lymphocytes enter the G1 stage of the cell cycle before going on to divide. Activated lymphocytes are larger (10 to 12 μm in diameter), have more cytoplasm and organelles and increased amounts of cytoplasmic RNA, and are called large lymphocytes or lymphoblasts (see Fig. 2.10). These changes require more energy and substrates for biosynthetic activities. The recently activated lymphocytes use aerobic glycolysis for energy and the tricarboxylic acid (TCA) cycle to generate the intermediary metabolites needed for new synthesis of proteins, lipids, and nucleic acids.

Some of these activated lymphocytes differentiate into effector lymphocytes that have the ability to produce molecules capable of eliminating foreign antigens. Effector T lymphocytes include CD4$^+$ helper T cells and CD8$^+$ CTLs, and effector B lymphocytes are antibody-secreting cells, mainly plasmablasts and plasma cells. Helper T cells activate B lymphocytes, macrophages, and DCs by secreted cytokines that bind to receptors on these cells, and surface molecules, such as CD40 ligand (CD154), which engages CD40 on other cells. CTLs have cytoplasmic granules filled with proteins that, when released, kill the cells that the CTLs recognize, which are usually virus-infected cells or tumor cells. Both CD4$^+$ and CD8$^+$ effector T cells usually express surface proteins indicative of recent activation, including CD25 (a component of the receptor for the T cell growth factor IL-2), and altered patterns of molecules that mediate migration (selectins, integrins, and chemokine receptors, discussed in Chapter 3). The majority of differentiated effector T lymphocytes migrate from secondary lymphoid organs, where they were generated, into tissue sites of infections, and are short lived.

Many antibody-secreting B cells are morphologically identifiable in stained tissue sections as **plasma cells.** They have characteristic nuclei placed eccentrically in the cell with the chromatin distributed around the nuclear membrane in a cartwheel pattern; abundant cytoplasm containing dense, rough endoplasmic reticulum that is the site where antibodies (and other secreted and membrane proteins) are synthesized; and distinct perinuclear Golgi complexes, where antibody molecules are post-translationally modified to their final forms and packaged for secretion (Fig. 2.11). It is estimated that half or more of the messenger RNA in these cells codes for antibody proteins and a single plasma cell can secrete thousands of antibody molecules per second. Plasma cells develop in lymphoid organs and at sites of infection, and some of them migrate to the bone marrow or mucosal tissues, where they may live and secrete antibodies for long periods after the immune response is induced and even after the antigen is eliminated. **Plasmablasts** are antibody-secreting cells with features of plasma cells but that are capable of proliferation; they are found in the circulation and can be identified by expression of CD19 and low levels of the typical B cell marker CD20 compared with naive and memory B cells. Within a week after an infection, a large number of plasmablasts can be detected in the blood, which secrete IgM, IgG, or IgA antibodies and are derived from naive or memory B cells that were recently activated in secondary lymphoid organs. Some of these circulating plasmablasts are likely in transit from the lymphoid organs to the bone marrow and mucosal tissues, where they will remain as long-lived plasma cells.

Memory Lymphocytes

Memory cells are generated during infections but may survive in a functionally quiescent or slowly cycling state for months or years after the microbe is eliminated. Some memory cells recirculate between blood and lymphoid tissues, similar to naive T cells, and others remain within nonlymphoid tissues without reentering the blood for long periods. Memory lymphocytes can be identified by their expression of surface proteins that distinguish them from naive and recently activated effector lymphocytes, although it is still not clear which of these surface proteins are definitive markers of memory populations (see Table 2.5). Memory T cells, like naive but not effector T cells, express high levels of the IL-7 receptor. Memory T cells also express molecules that regulate their migration into and out of lymphoid organs or tissue sites of infection, and these vary depending on the subset (see Chapter 3). In humans, most naive T cells express a 200-kD isoform of a surface molecule called CD45 that contains a segment encoded by an exon designated A and is therefore called CD45RA (for restricted A). In contrast, most activated and memory T cells express a 180-kD isoform of CD45 in which the A exon RNA has been spliced out; this isoform is called CD45RO. However, this way of distinguishing naive from memory T cells is not perfect, and interconversion between CD45RA$^+$ and CD45RO$^+$ populations has been documented.

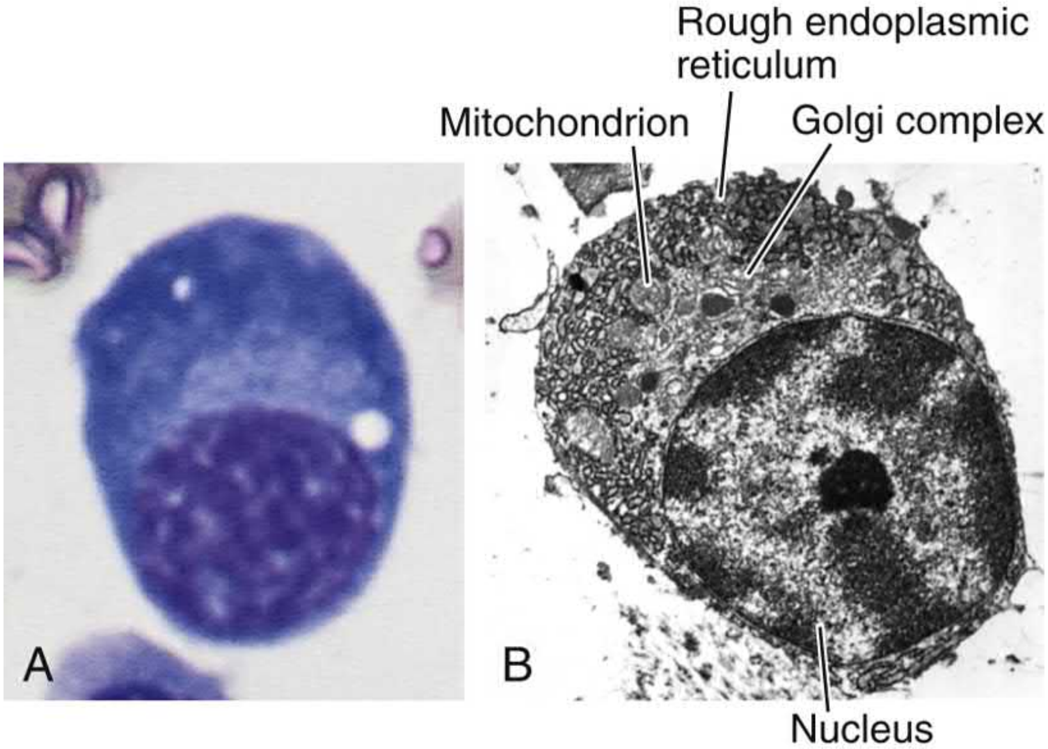

Rough endoplasmic reticulum
Mitochondrion
Golgi complex
A
B
Nucleus

FIGURE 2.11 Morphology of plasma cells. **A,** Light micrograph of a plasma cell in tissue. **B,** Electron micrograph of a plasma cell.

Courtesy of Dr. Noel Weidner, Department of Pathology, University of California, San Diego, California.

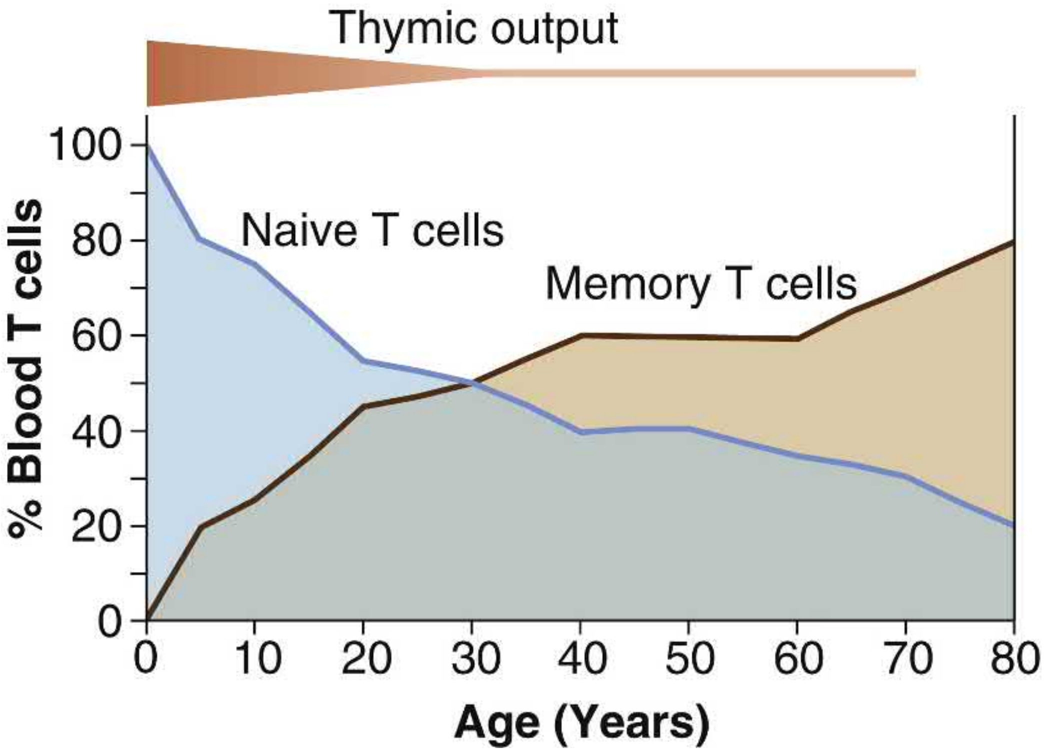


FIGURE 2.12 Change in proportions of naive and memory T cells with age. The proportions of naive and memory T cells are based on data from multiple healthy individuals. The estimate of thymic output is an approximation.

Courtesy of Dr. Donna L. Farber, Columbia University College of Physicians and Surgeons, New York, New York.

The frequency of memory cells increases with age because individuals are continually exposed to foreign antigens, such as environmental microbes. Memory T cells make up less than 5% of peripheral blood T cells in a newborn but 50% or more in an adult (Fig. 2.12). As individuals age, the gradual accumulation of memory cells compensates for the reduced output of new naive T cells from the thymus, which involutes after puberty, as discussed later.

Memory B lymphocytes may express certain classes (isotypes) of membrane Ig, such as IgG, IgE, or IgA, as a result of isotype switching, whereas naive B cells express only IgM and IgD (see Chapters 5 and 12). In humans, CD27 expression is a marker for memory B cells.

Memory cells are heterogeneous and consist of subsets that differ especially with respect to their location and migratory properties. Memory T and B cells will be discussed further in Chapters 9 and 12, respectively.

The distinguishing features of naive, effector, and memory lymphocytes reflect different programs of gene expression that are regulated by transcription factors and stable epigenetic changes, including histone methylation and acetylation and chromatin remodeling. For example, a transcription factor called Kruppel-like factor 2 (KLF2) is required for maintenance of the naive T cell phenotype. The phenotypes of functionally different types of CD4$^+$ effector T cells, called Th1, Th2, and Th17 cells, depend on transcription factors T-BET, GATA3, and RORγT, respectively, as well as epigenetic changes in cytokine gene loci (see Chapter 10). Other transcription factors are required for maintaining the phenotypes of memory B and T cells. Our understanding of the molecular determinants of lymphocyte phenotype is still incomplete and evolving.

Natural Killer Cells and Cytokine-Secreting Innate Lymphoid Cells

The innate immune system includes several developmentally related subsets of bone marrow–derived cells with lymphoid morphology and effector functions similar to those of T cells but lacking T cell antigen receptors. The major functions of these cells are to provide early defense against infectious pathogens, to recognize stressed and damaged host cells and help to eliminate these cells, and to influence the nature of the subsequent adaptive immune response. Natural killer (NK) cells have cytotoxic activity similar to that of CD8$^+$ CTLs. They circulate in the blood and are present in various lymphoid tissues. Innate lymphoid cells (ILCs) produce cytokines similar to those secreted by CD4$^+$ helper T cells. ILCs can be grouped into three major subsets based on the cytokines they secrete, analogous to the three subsets of CD4$^+$ helper T cells, described in Chapter 10. ILCs are rare in the blood and are present mostly in tissues, especially mucosal tissues such as the lung and intestines. The common lymphoid progenitor in the bone marrow that gives rise to T and B lymphocytes also gives rise to a precursor of both NK cells and ILCs, and NK cells and ILCs share expression of several lineage-specific markers and transcription factors. Lymphoid tissue–inducer cells are a type of ILC that produces the cytokines lymphotoxin and TNF and are essential for the formation of organized secondary lymphoid tissues, described later in this chapter. NK cells and ILCs are described in more detail in Chapter 4.

Anatomy and Functions of Lymphoid Tissues

The primary lymphoid organs, also called generative or central lymphoid organs, include the bone marrow and thymus, and are the sites where lymphocytes first express antigen receptors and attain phenotypic and functional maturity (see Fig. 2.8) **.** B lymphocytes mature partially in the bone marrow; enter the circulation; migrate to the spleen, where they complete their maturation; and then populate secondary lymphoid organs. T lymphocytes mature in the thymus, then enter the circulation, and migrate to secondary lymphoid organs. Two important functions shared by the generative organs are to provide growth factors and other molecular signals needed for lymphocyte maturation and to present self antigens for selection of maturing lymphocytes (see Chapter 8).

Secondary (or peripheral) lymphoid organs, including the lymph nodes, spleen, and components of the mucosal immune system, are where lymphocyte responses to foreign antigens are initiated and develop (see Fig. 2.8) **.** These organs are anatomically organized in ways that optimize the cellular interactions necessary for initiation of adaptive immune responses. Lymphocytes and APCs are localized and concentrated in anatomically defined areas of these organs, which are also the sites where foreign antigens are transported and concentrated. This ensures that antigens and antigen-specific naive lymphocytes can come together to initiate adaptive immune responses. The anatomy of lymphoid organs also enables T cells and B cells to interact after they are activated by antigens. As we will discuss in Chapter 3, many lymphocytes constantly recirculate and exchange among the circulation, secondary lymphoid organs, and tissues.

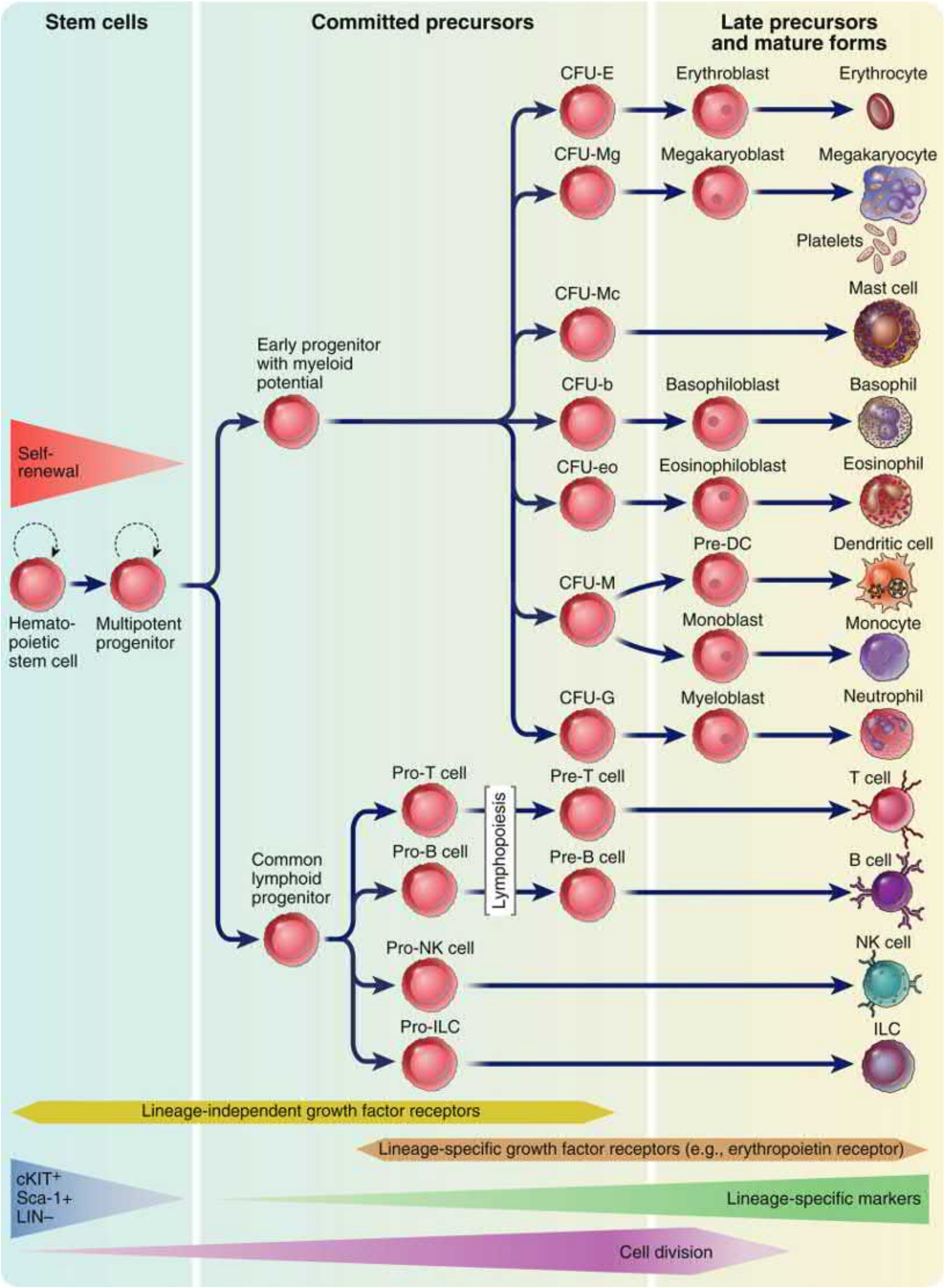
Bone Marrow

The bone marrow is the site of generation of circulating blood cells, including red blood cells, granulocytes, and monocytes, and the site of B cell maturation. The generation of all blood cells, called **hematopoiesis** (Fig. 2.13), occurs initially during fetal development in blood islands of the yolk sac and the para-aortic mesenchyme, then shifts to the liver between the third and fourth months of gestation, and finally shifts to the bone marrow. At birth, hematopoiesis takes place in the bones throughout the skeleton, but it becomes increasingly restricted to the marrow of the flat bones, so that by puberty, hematopoiesis occurs mostly in the sternum, vertebrae, iliac bones, and ribs. The red marrow that is found in these bones consists of a spongelike reticular framework located between long bony trabeculae. The spaces in this framework contain a network of blood-filled sinusoids lined by endothelial cells attached to a discontinuous basement membrane. Outside the sinusoids are clusters of the precursors of blood cells in various stages of development, as well as fat cells. The blood cell precursors mature and then migrate through the sinusoidal basement membrane and between endothelial cells to enter the vascular circulation. When the bone marrow is compromised or there is an exceptional demand for production of new blood cells, the liver and spleen often become sites of extramedullary hematopoiesis.

Red blood cells, granulocytes, monocytes, DCs, mast cells, platelets, B and T lymphocytes, and ILCs all originate from a common HSC in the bone marrow (see Fig. 2.13). HSCs are multipotent, meaning that a single HSC can generate all different types of mature blood cells. HSCs are also self-renewing because each time they divide, at least one daughter cell maintains the properties of a stem cell and the other can differentiate along a particular lineage (called asymmetric division). HSCs can be identified by the presence of surface markers, including the proteins CD34 and c-KIT, and the absence of lineage-specific markers that are expressed in mature cells. HSCs are maintained within specialized microscopic anatomic niches in the bone marrow. In these locations, nonhematopoietic stromal cells provide contact-dependent signals and growth factors required for continuous cycling of the HSCs. The common myeloid-lymphoid progenitor gives rise to some myeloid cells and to committed precursors of T cell, B cell, and NK/ILC lineages. The common myeloid-megakaryocyte-erythroid progenitors give rise to committed precursors of the erythroid, megakaryocytic, granulocytic, and monocytic lineages, which give rise, respectively, to mature red blood cells, platelets, granulocytes (neutrophils, eosinophils, basophils), and monocytes. As discussed earlier, most DCs arise from a precursor that also gives rise to monocytes. Immature mast cell progenitors arise from a common granulocyte/monocyte precursor, leave the bone marrow, and mature into mast cells in peripheral tissues.

The proliferation and maturation of precursor cells in the bone marrow are stimulated by cytokines. Many of these cytokines are called **colony-stimulating factors** because they were originally assayed by their ability to stimulate the growth and development of various leukocytic or erythroid colonies from marrow cells. Hematopoietic cytokines are produced by stromal cells and macrophages in the bone marrow, thus providing the local environment for hematopoiesis. They are also produced by antigen-stimulated T lymphocytes and cytokine-activated or microbe-activated macrophages, providing a mechanism for increasing leukocyte production when needed for immune and inflammatory reactions and for replenishing leukocytes that may be consumed during these reactions. The names and properties of the major hematopoietic cytokines are listed in Table 2.7.

In addition to self-renewing stem cells and their differentiating progeny, the marrow contains numerous long-lived antibody-secreting plasma cells. These cells are generated in secondary lymphoid organs as a consequence of stimulation of B cells by antigens and helper T cells, and then migrate to the bone marrow. In addition, some long-lived memory T lymphocytes migrate to and may reside in the bone marrow.



Stem cells
Committed precursors
Late precursors and mature forms
CFU-E
Erythroblast
Erythrocyte
CFU-Mg
Megakaryoblast
Megakaryocyte
Platelets
CFU-Mc
Mast cell
Early progenitor with myeloid potential
CFU-b
Basophiloblast
Basophil
Self-renewal
CFU-eo
Eosinophiloblast
Eosinophil
Pre-DC
Dendritic cell
CFU-M
Monoblast
Monocyte
Hemato-poietic stem cell
Multipotent progenitor
CFU-G
Myeloblast
Neutrophil
Pro-T cell
Pre-T cell
T cell
Lymphopoiesis
Common lymphoid progenitor
Pro-B cell
Pre-B cell
B cell
Pro-NK cell
NK cell
Pro-ILC
ILC
Lineage-independent growth factor receptors
Lineage-specific growth factor receptors (e.g., erythropoietin receptor)
cKIT+
Sca-1+
LIN–
Lineage-specific markers
Cell division

FIGURE 2.13 Hematopoiesis.The development of the major lineages of blood cells is depicted in this hematopoietic tree. The principal cytokines that drive the maturation of different lineages are described in Table 2.7. The development of lymphocytes is described later in this chapter and

in Chapter 8. *CFU,* Colony-forming unit; *CFU-Mc,* CFU mast cell; *CFU-b,* CFU B cell; *CFU-eo,* CFU eosinophil; *CFU-G,* CFU granulocytes; *CFU-M,* CFU macrophages; *DC,* dendritic cell; *ILCs,* innate lymphoid cells; *NK,* natural killer.

TABLE 2.7

Cytokine	Size	Principal Cellular Sources	Principal Immature Cell Targets	Principal Cell Populations Induced
Stem cell factor (c-KIT ligand)	24 kD	Bone marrow stromal cells	HSCs	All
Interleukin-7 (IL-7)	25 kD	Fibroblasts, bone marrow stromal cells	Immature lymphoid progenitors	T lymphocytes
Interleukin-3 (IL-3)	20–26 kD	T cells	Immature progenitors	All
GM-CSF	18–22 kD	T cells, macrophages, endothelial cells, fibroblasts	Immature and committed myeloid progenitors, mature macrophages	Granulocytes and monocytes, macrophage activation
M-CSF	Dimer of 70–90 kD; 40-kD subunits	Macrophages, endothelial cells, bone marrow cells, fibroblasts	Committed progenitors	Monocytes
G-CSF	19 kD	Macrophages, fibroblasts, endothelial cells	Committed granulocyte progenitors	Granulocytes
FLT-3 ligand	30 kD	Bone marrow stromal cells	HSCs, DC and B cell progenitors	Classical and plasmacytoid DCs, B cells

DC, Dendritic cells; *G-CSF,* granulocyte colony-stimulating factor; *GM-CSF,* granulocyte-monocyte colony-stimulating factor; *HSCs,* hematopoietic stem cell; *IL,* Interleukin; *M-CSF,* monocyte colony-stimulating factor.

Thymus

The thymus is the site of T cell maturation. It is a bilobed organ situated in the anterior mediastinum, which involutes after puberty so that it is not detectable in adults. Each lobe is divided into multiple lobules by fibrous septa, and each lobule consists of an outer cortex and an inner medulla (Fig. 2.14). The cortex contains a dense collection of bone marrow–derived T lymphocytes, and the lighter staining medulla is more sparsely populated with lymphocytes. The medulla also contains macrophages and DCs. Scattered throughout the thymus are epithelial cells, which have abundant cytoplasm. The epithelial component of the thymus is derived from invaginations of the ectoderm in the developing neck and chest of the embryo, forming structures called branchial pouches. Thymic cortical epithelial cells produce IL-7, which is required early in T cell development, and these cells also present self antigens to developing T cells during their maturation. A different type of epithelial cell found only in the medulla, called medullary thymic epithelial cells (MTECs), plays a special role in presenting self antigens to developing T cells and causing their elimination. This is one mechanism for ensuring that the immune system remains tolerant to self antigens and is discussed in detail in Chapter 15. In the medulla, there are structures called Hassall's corpuscles, which are composed of tightly packed whorls of epithelial cells that may be remnants of degenerating cells. The thymus has a rich vascular supply and efferent lymphatic vessels that drain into mediastinal lymph nodes.

An inherited disorder of T cell immunity caused by failure of development of the thymus is called the **DiGeorge syndrome.** These patients suffer from T cell deficiency because of a chromosomal deletion that eliminates genes required for thymus development (see Chapter 21). In the nude mouse strain, which has been widely used in immunology research, a mutation in the gene encoding a transcription factor called Tbx1 causes a failure of differentiation of certain types of epithelial cells that are required for normal development of the thymus and hair follicles. Consequently, these mice lack T cells and hair; these mice have been used for research studies analyzing the consequences of T cell deficiency.

The lymphocytes in the thymus, also called **thymocytes,** are T cells at various stages of maturation. The most immature cells from the bone marrow enter the thymus via the blood, and their maturation begins in the cortex. As thymocytes mature, they migrate toward the medulla, so the medulla contains mostly mature T cells. Only mature naive T cells exit the thymus and enter the blood and peripheral lymphoid tissues. The details of thymocyte maturation are described in Chapter 8. The thymus involutes after puberty; hence, the output of mature T cells decreases progressively with age.

Lymphatic System

The lymphatic system consists of specialized vessels, called lymphatics, that drain fluid from tissues, and lymph nodes interspersed along the vessels (Fig. 2.15)**.** Lymphatics are essential for tissue fluid homeostasis and for immune responses. Interstitial fluid is constantly formed in all vascularized tissues by movement of a filtrate of plasma out of capillaries, and the rate of local formation can increase dramatically when tissue is injured or infected. The skin, epithelia, and parenchymal organs contain numerous lymphatic capillaries that absorb this fluid from spaces between tissue cells. Lymphatic capillaries are blind-ended vascular channels lined by overlapping lymphatic endothelial cells without the tight intercellular junctions or continuous basement membrane that are typical of blood vessels. Lymphatic vessels are attached to the extracellular matrix by elastin fibers, which serve to pull them open when there is excess fluid accumulation and tissue swelling. These vessels permit free uptake of the interstitial fluid, and the overlapping arrangement of the endothelial cells and one-way valves within their lumens prevent backflow of the fluid. The absorbed fluid, called **lymph,** is pumped into convergent, progressively larger lymphatic vessels by the contraction of perilymphatic smooth muscle cells and the pressure exerted by movement of the musculoskeletal tissues. These vessels merge into afferent lymphatics that drain into lymph nodes, and the lymph drains out of the nodes through efferent lymphatics. Because lymph nodes are connected in series by lymphatics, an efferent lymphatic exiting one node may serve as the afferent vessel for another. The efferent lymph vessel at the end of a lymph node chain joins other lymph vessels, eventually culminating in a large lymphatic vessel called the thoracic duct. Lymph from the thoracic duct is emptied into the superior vena cava, thus returning the fluid to the blood stream. Lymphatics from the right upper trunk, right arm, and right side of the head drain into the right lymphatic duct, which also drains into the superior vena cava. Approximately 2 liters of lymph are normally returned to the circulation each day, and disruption of the lymphatic system by tumors or some parasitic infections may lead to severe tissue swelling because of fluid accumulation.

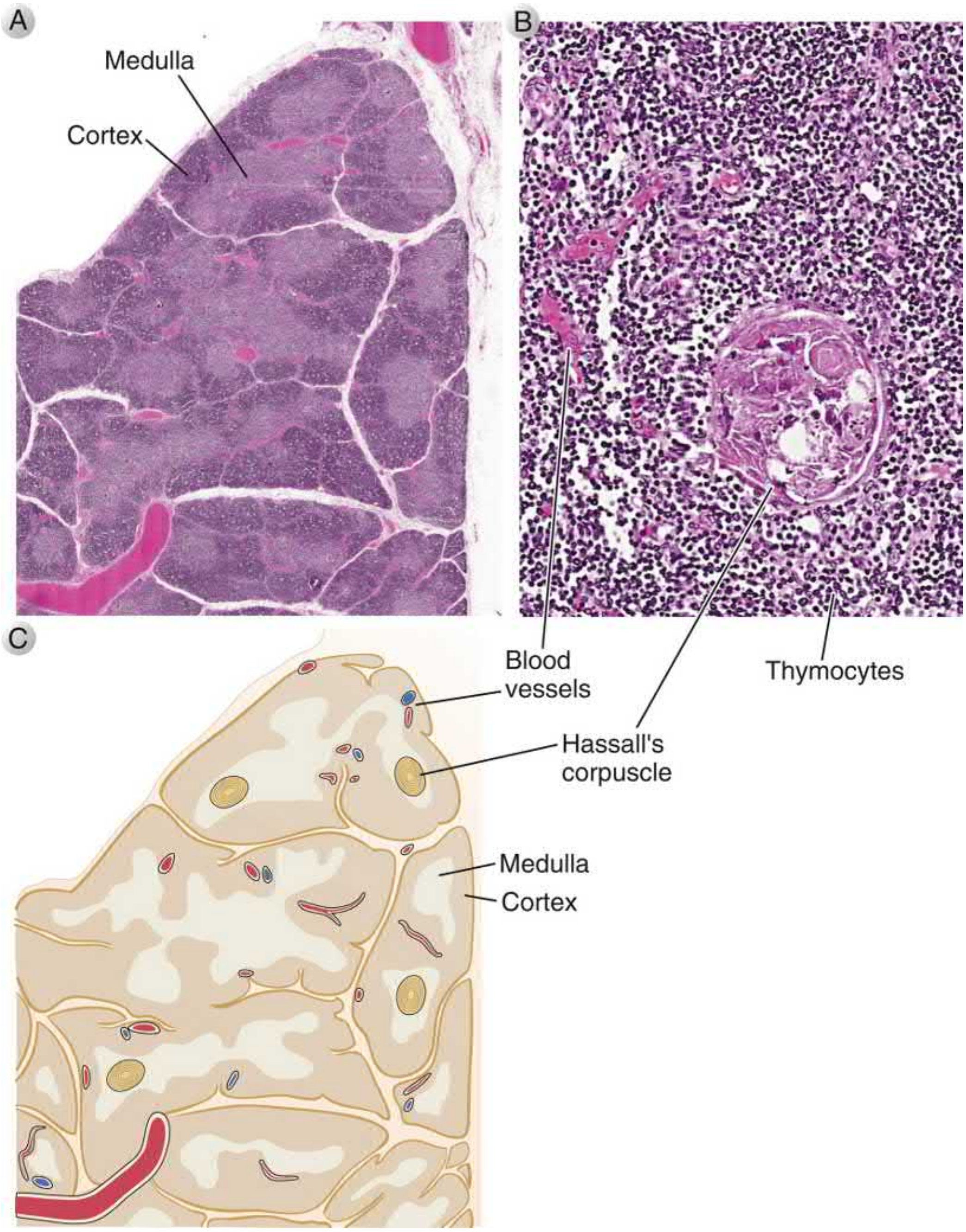

A
Medulla
Cortex
B
Blood vessels
Thymocytes
Hassall's corpuscle
C
Medulla
Cortex

FIGURE 2.14 Morphology of the thymus. **A,** Low-power light micrograph of a lobe of the thymus showing the cortex and medulla. The darker stained outer cortex and paler inner medulla are apparent. **B,** High-power light micrograph of the thymic medulla. The numerous small *blue*-staining cells are developing T cells called *thymocytes,*

and the larger *pink* structure is a Hassall’s corpuscle, uniquely characteristic of the thymic medulla but whose function is poorly understood. **C,** Schematic diagram of the thymus illustrating a portion of a lobe divided into multiple lobules by fibrous trabeculae.

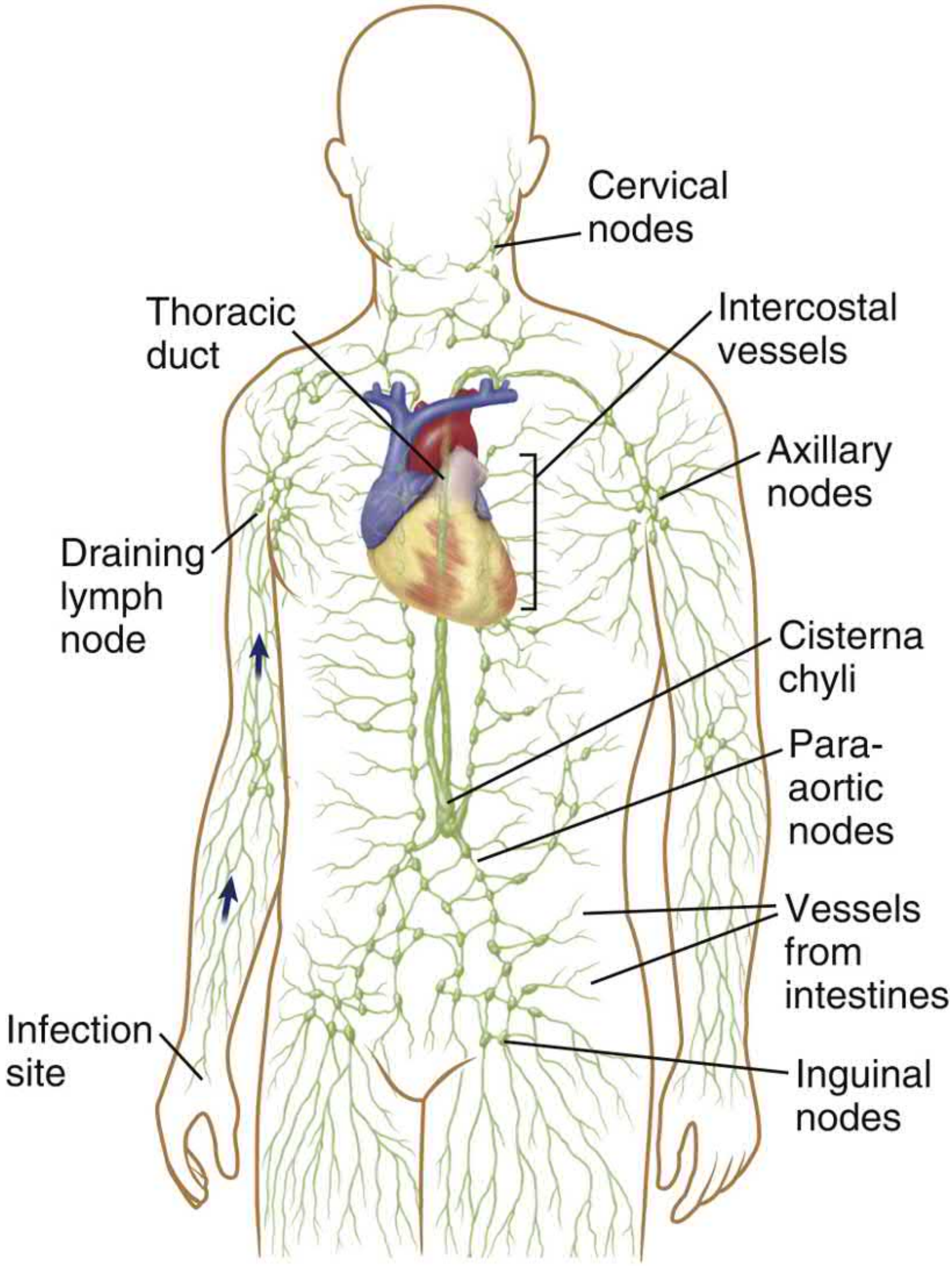

Cervical nodes
Thoracic duct
Intercostal vessels
Axillary nodes
Draining lymph node
Cisterna chyli
Para-aortic nodes
Vessels from intestines
Infection site
Inguinal nodes

FIGURE 2.15 The lymphatic system. The major lymphatic vessels, which drain into the inferior vena cava (and superior vena cava, not shown), and collections of lymph nodes are illustrated. Antigens are captured from a site of infection and the draining lymph node to which these antigens

are transported and where the immune response is initiated.

Lymphatic vessels collect microbial antigens from their portals of entry and deliver the antigens to lymph nodes, where they can stimulate adaptive immune responses. Microbes enter the body most often through the skin and the gastrointestinal and respiratory tracts. All of these tissues are lined by epithelial barriers that contain DCs, and all are drained by lymphatic vessels. The DCs capture microbial antigens and enter lymphatic vessels through gaps in the basement membrane. The migration of DCs into lymphatics, and then to specific locations in lymph nodes, is guided by chemokines produced by lymphatic endothelial cells and by stromal cells in the node, discussed in detail in Chapter 6. Other microbes as well as soluble antigens may enter lymphatics independently of DCs.

Lymph Nodes

Lymph nodes are encapsulated, vascularized secondary lymphoid organs with anatomic features that favor the initiation of adaptive immune responses to antigens carried from tissues by lymphatics (Fig. 2.16) **.** Lymph nodes located along lymphatic vessels act as filters that sample the lymph for soluble and DC-associated antigens. The captured antigens can then be seen by cells of the adaptive immune system (see Chapter 6). There are approximately 500 lymph nodes in the human body. A lymph node is surrounded by a fibrous capsule, beneath which is a system of sinuses lined by reticular cells, cross-bridged by fibrils of collagen and other extracellular matrix proteins, and filled with lymphocytes, macrophages, DCs, and other cell types. Afferent lymphatics empty into the subcapsular sinus, and lymph may drain from there directly into the connected medullary sinus and then out of the lymph node through the efferent lymphatics. Macrophages in the subscapular sinus provide an important function of phagocytosing and removing infectious organisms, which they can recognize by a wide variety of cell surface receptors. Beneath the inner floor of the subcapsular sinus is the lymphocyte-rich cortex. The outer cortex contains aggregates of cells called **follicles** that are populated mainly by B lymphocytes. The cortex around the follicles, called the parafollicular cortex, paracortex, or T cell zone, is organized into cords, with abundant extracellular matrix proteins and fibers, and is populated mainly by T lymphocytes.

The development of lymph nodes, as well as of other secondary lymphoid organs, depends on lymphoid tissue–inducer cells and the coordinated actions of several cytokines, chemokines, and transcription factors. During fetal life, lymphoid tissue–inducer cells, which are a subset of ILCs discussed earlier, stimulate the development of lymph nodes and other secondary lymphoid organs. This function is mediated by various proteins expressed by the inducer cells, the most thoroughly studied being the membrane bound heterotrimeric molecule LTαβ2. Mice with genetic deletions in either LT α or β do not develop lymph nodes or secondary lymphoid tissues in the gut. Splenic white pulp development is also disorganized in these mice. Lymphotoxin produced by the inducer cells stimulates stromal cells in different locations of a developing secondary lymphoid organ to secrete chemokines that help to organize the structure of the lymphoid organs.

Anatomic Organization of B and T Lymphocytes

B and T lymphocytes are sequestered in distinct regions of the cortex of lymph nodes (Fig. 2.17) **.** B cells are found mainly in the follicles in the cortex. Some follicles contain central areas called **germinal centers,** which stain lightly with commonly used histologic stains. Follicles without germinal centers, called primary follicles, contain mostly mature, naive B lymphocytes. Follicles with germinal centers, called secondary follicles, contain activated B cells. Germinal centers develop in response to antigenic stimulation and are sites of remarkable B cell proliferation, selection of B cells producing high-affinity antibodies, and generation of memory B cells and long-lived plasma cells. Each germinal center consists of a dark zone packed with proliferating B cells called centroblasts and a light zone containing cells called centrocytes that have stopped proliferating and are being selected to survive and differentiate further. The germinal center reaction during humoral immune responses is described in Chapter 12.

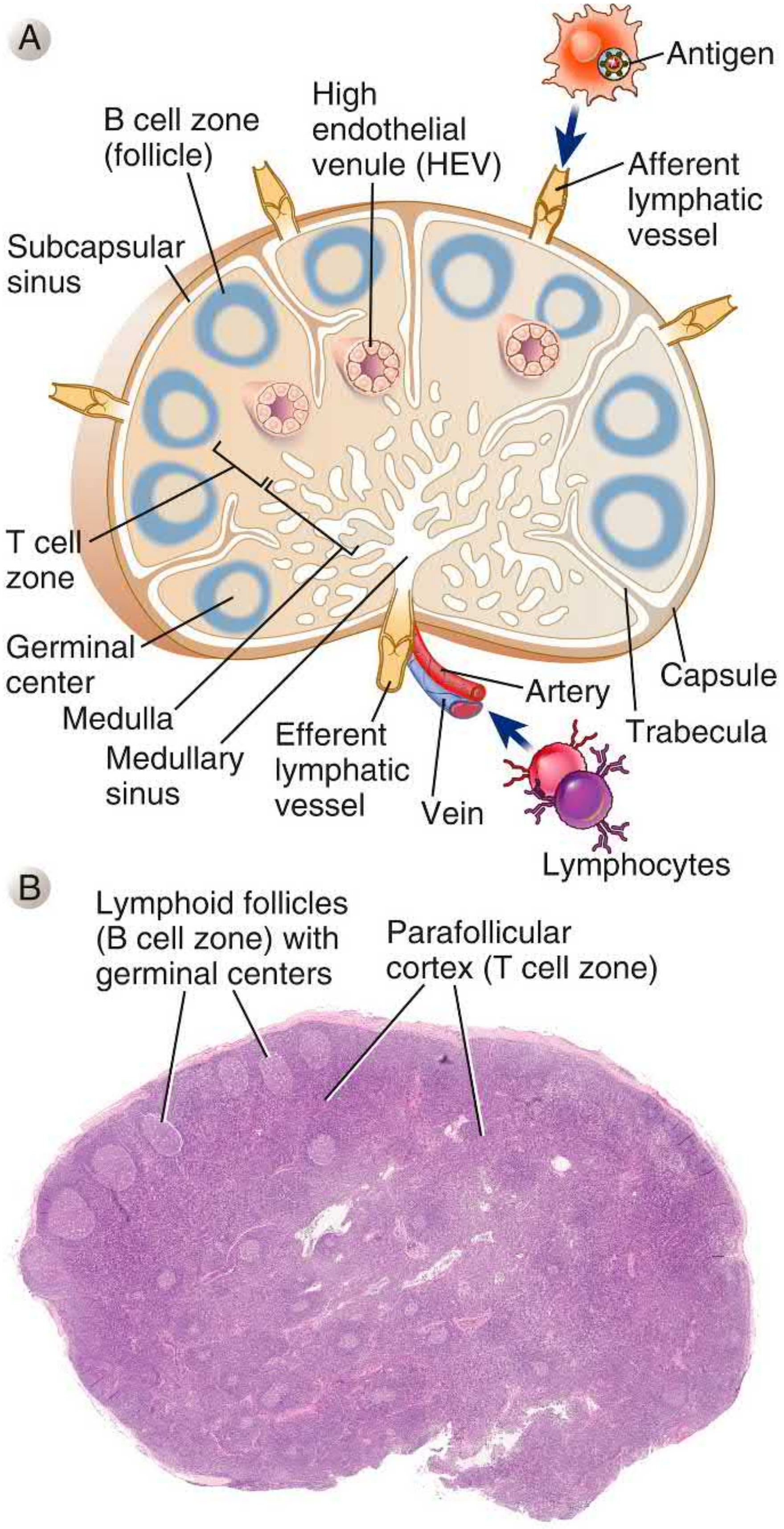

A
Antigen
High endothelial venule (HEV)
B cell zone (follicle)
Afferent lymphatic vessel
Subcapsular sinus
T cell zone
Germinal center
Medulla
Medullary sinus
Efferent lymphatic vessel
Vein
Artery
Capsule
Trabecula
Lymphocytes
B
Lymphoid follicles (B cell zone) with germinal centers
Parafollicular cortex (T cell zone)

FIGURE 2.16 Morphology of a lymph node. **A,** Schematic diagram of a lymph node illustrating the T cell–rich and B cell–rich zones and the routes of entry of lymphocytes and antigen (shown captured by a dendritic cell). **B,** Light micrograph of a lymph node illustrating the T cell and B cell zones.

Courtesy of Robert Oghami, MD, PhD, and Kaushik Sridhar, Department of Pathology, University of California San Francisco.

T lymphocytes are located mainly beneath and more central to the follicles, in the paracortical cords. Naive T cells enter the T cell zones through specialized cortical blood vessels called **high endothelial venules** (HEVs), as described in detail in Chapter 3, and T cells are densely packed around the HEVs. Most (~70%) of the cortical T cells are $CD4^+$ helper T cells, intermingled with fewer $CD8^+$ cells. These proportions can change dramatically during the course of an infection. For example, during a viral infection, there may be a marked increase in $CD8^+$ T cells. DCs are also concentrated in the T cell zones of the lymph nodes, where they can present antigens to the T cells.

The anatomic segregation of B and T lymphocytes in distinct areas of the node is dependent on chemokines (chemoattractant cytokines), that are secreted by specialized cells located in each area and that direct the migration of the lymphocytes (see Fig. 2.17) **.** These specialized cells are **fibroblastic reticular cells** (FRCs) in the T cell zones and follicular dendritic cells (FDCs) in the follicles. FRCs are mesenchymally derived myofibroblasts that drive formation of secondary lymphoid organs during embryonic development and contribute in multiple ways to the functions of these organs. There are several subtypes of FRCs located in different places within secondary lymphoid organs. Most FRCs in lymph nodes can be identified by expression of podoplanin, a glycoprotein that may facilitate adhesion of the FRCs to the stromal reticular (connective tissue) scaffold. Some types of FRC that play central roles in maintaining the structure and functions of lymph nodes include marginal reticular cells that form the subcapsular sinus floor, perivascular reticular cells that form layers around HEVs, T cell zone FRCs that direct movement of T cells and DCs within the lymph node, and B cell zone FRCs that direct movement of B and T cells in and out of B cell follicles. FDCs are derived from an FRC precursor, and play essential roles in maintaining follicle structure as well as in B cell activation (see Chapter 12).

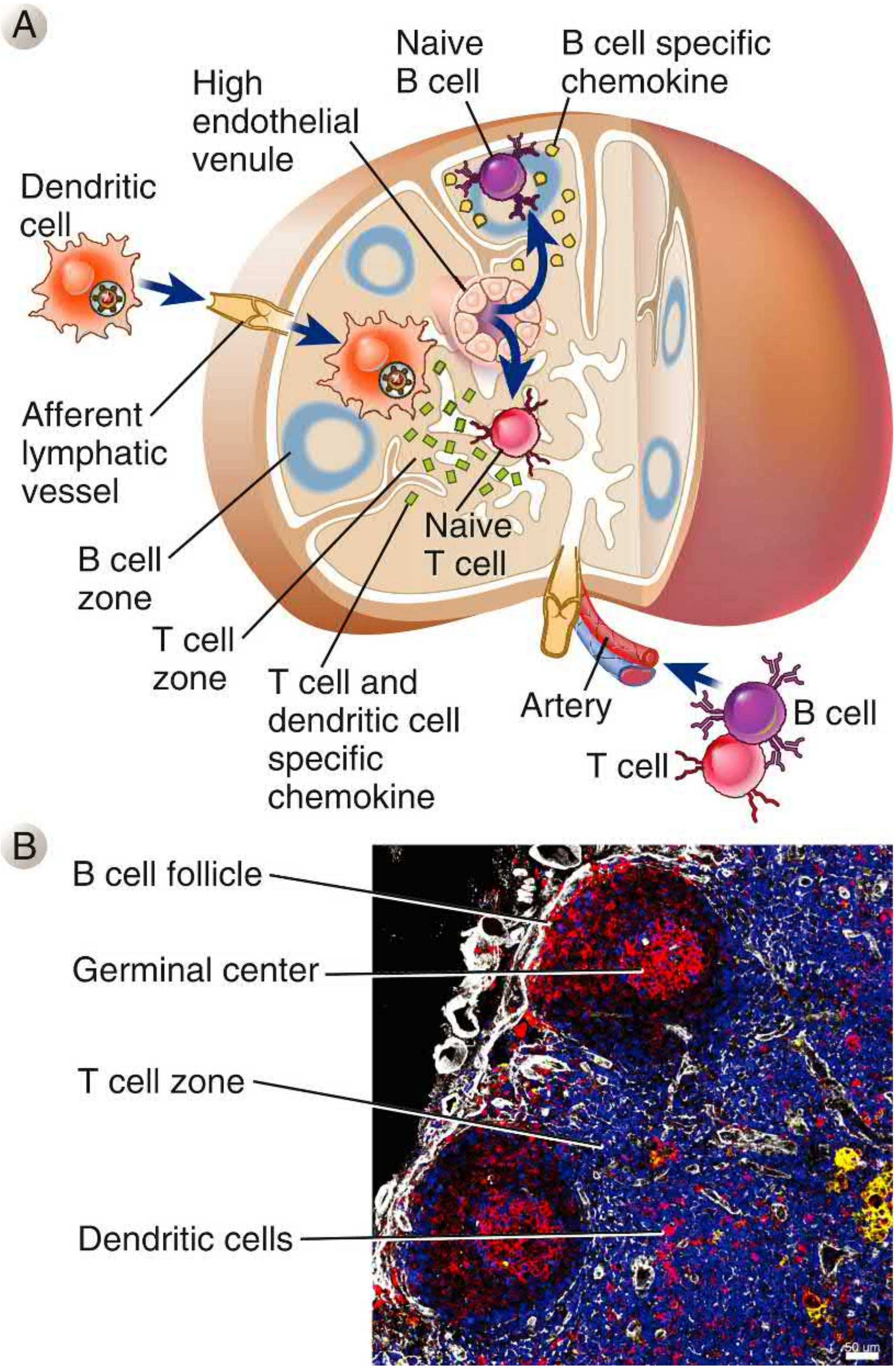

A
Naive B cell
B cell specific chemokine
High endothelial venule
Dendritic cell
Afferent lymphatic vessel
B cell zone
T cell zone
T cell and dendritic cell specific chemokine
Naive T cell
Artery
B cell
T cell
B
B cell follicle
Germinal center
T cell zone
Dendritic cells
50 µm

FIGURE 2.17 Segregation of B cells and T cells in a lymph node. **A,** The schematic diagram illustrates

the path by which naive T and B lymphocytes migrate to different areas of a lymph node. The naive lymphocytes enter the node through an artery, leave the circulation by moving across the wall of the high endothelial venule (HEV), and then the B and T cells migrate to different zones of the lymph node drawn by chemokines that are produced in these areas and bind selectively to chemokine receptors specific for each cell type. Also shown is the migration of dendritic cells (DCs), which pick up antigens from the sites of antigen entry, enter through afferent lymphatic vessels, and migrate to the T cell–rich areas of the node. **B,** In this section of a lymph node, the B lymphocytes, located in two follicles, are stained *red;* the T cells, in the parafollicular cortex, are *blue.* Dendritic cells, in the parafollicular cortex, are also stained *red.* The method used to stain these cells is called immunofluorescence (see Appendix III for details).

The anatomic segregation of T and B cells is also seen in the spleen (see Fig. 2.19). (Courtesy of Andrea Radtke, PhD, laboratory of Ronald N. Germain, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Chemokines are a large family of 8- to 10-kD cytokines that are involved in cell motility functions in development, maintenance of tissue architecture, and immune and inflammatory responses. Chemokines bind to receptors on lymphocytes and other cells and stimulate the cells to move up concentration gradients of the chemokine. We will discuss the properties of chemokines and their receptors in Chapter 3. The production of particular chemokines in different areas of secondary lymphoid organs and the expression of receptors for these chemokines determine where B and T cells reside in these organs. Naive T cells express a receptor called CCR7 that binds the chemokines CCL19 and CCL21, which are produced by T cell zone FRCs. These chemokines promote naive T cell movement from the blood, through the wall of the HEVs, into the T cell zone. DCs that are activated by microbes also express CCR7, and lymphatic endothelial cells express CCL21; this is why DCs enter the node through lymphatics, and why they migrate to the same area of the node as do naive T cells (see Chapter 6). Naive B cells express low levels of CCR7 and higher levels of another chemokine receptor, CXCR5, which recognizes a chemokine, CXCL13, produced by B cell zone FRCs and FDCs. Thus, circulating naive B cells that enter lymph nodes, also through HEVs, are attracted into the follicles. The functions of chemokines in regulating where lymphocytes are located in lymphoid organs and in the formation of these organs have been established by numerous studies in mice. For example, CXCR5 knockout mice lack B cell–containing follicles in lymph nodes and spleen, and CCR7 knockout mice lack T cell zones.

The anatomic segregation of B and T cells ensures that each lymphocyte population is in close contact with the appropriate APCs (i.e., B cells with FDCs and T cells with DCs) **.** Furthermore, because of this precise segregation, B and T lymphocyte populations are kept apart until it is time for them to interact in a functional way. As we will see in Chapters 9 and 12, after stimulation by protein antigens, B and T cells change their expression of chemokine receptors and begin to migrate toward one another in response to signals from chemokines and other mediators. Activated T cells either migrate toward follicles to help B cells or exit the node and enter the circulation. Activated B cells migrate into germinal centers and, after differentiation into plasma cells, may home to the bone marrow.

T cell zone FRCs maintain lymph node structure and function by forming a network of specialized tube-like structures called FRC conduits (Fig. 2.18)**.** These conduits range in diameter from 0.2 to 3 μM and contain organized arrays of extracellular matrix molecules secreted by the FRCs, including parallel bundles of collagen fibers embedded in a meshwork of fibrillin microfibers, all tightly surrounded by a basement membrane produced by a sleeve of

FRCs. The FRC conduits display chemokines on their surfaces and act as tracks along which T cells and DCs migrate, responding to the chemokines. The FRC conduits also carry some antigens that enter the lymph nodes through afferent lymphatics into the T cell zone for access to antigen-presenting DCs. The conduits begin at the subcapsular sinus and extend to both medullary sinus lymphatic vessels and cortical HEVs.

Antigen Transport Through Lymph Nodes

Lymph-borne substances that enter the subcapsular sinus of the lymph node are sorted by molecular size and delivered to DCs, macrophages, and FDCs to initiate T and B cell responses. The floor of the subcapsular sinus is constructed in a way that permits cells in the sinus to contact or migrate into the underlying cortex but does not allow soluble molecules in the lymph to freely pass into the cortex. Microbes and high-molecular-weight antigens are taken up by sinus macrophages and presented to cortical B lymphocytes just beneath the sinus. This is the first step in antibody responses to these antigens. Low-molecular-weight soluble antigens are transported out of the sinus through the FRC conduits and passed to resident cortical DCs located adjacent to the conduits. The resident DCs extend processes between the cells lining the conduits and into the lumen and use these processes to capture and ingest the soluble antigens that have entered the conduits. This pathway of antigen delivery may play a role in initial T cell immune responses to some microbial antigens, but larger and sustained responses require delivery of antigens to the node by tissue DCs, as discussed in Chapter 6.

Spleen

The spleen is a highly vascularized organ whose major functions are to remove aging and damaged blood cells and particles (such as immune complexes and opsonized microbes) from the circulation and to initiate adaptive immune responses to blood-borne antigens. The spleen weighs approximately 150 g in adults and is located in the left upper quadrant of the abdomen. The splenic parenchyma is divided into red pulp, which is composed mainly of blood-filled vascular sinusoids, and lymphocyte-rich white pulp. Blood enters the spleen through a single splenic artery that pierces the capsule at the hilum and divides into progressively smaller branches that remain surrounded by protective and supporting fibrous trabeculae (Fig. 2.19). Some of the arteriolar branches of the splenic artery end in extensive vascular sinusoids that are filled with large numbers of erythrocytes and are lined by macrophages and other cells. The sinusoids end in venules that drain into the splenic vein, which carries blood out of the spleen and into the portal circulation. The red pulp macrophages serve as an important filter for the blood, removing microbes, damaged cells, and antibody-coated (opsonized) cells and microbes. Individuals lacking a spleen are susceptible to disseminated infections with encapsulated bacteria, such as pneumococci and meningococci. This is likely because such organisms are normally cleared by opsonization and phagocytosis and the spleen is an important site of antibody production, and both functions are defective in the absence of the spleen.

The white pulp contains the cells that mediate adaptive immune responses to blood-borne antigens. In the white pulp are many collections of densely packed lymphocytes, which appear as white nodules against the background of the vascular sinusoids. The white pulp is organized around central arteries, which are branches of the splenic artery distinct from the branches that form the vascular sinusoids. Several smaller branches of each central artery pass through the lymphocyte-rich area and drain into a marginal sinus. A region of specialized cells surrounding the marginal sinus, called the **marginal zone,** forms the boundary between the red pulp and white pulp. The architecture of the white pulp is analogous to the organization of lymph nodes, with segregated T cell and B cell zones. The central arteries are surrounded by cuffs of lymphocytes, most of which are T cells. Because of their anatomic location, morphologists call these T cell zones **periarteriolar lymphoid sheaths.** B cell–rich follicles occupy the space between the marginal sinus and the periarteriolar sheath. As in lymph nodes, the T cell areas in the spleen contain a network of conduits lined by FRCs. The marginal zone just outside the marginal sinus is a distinct region populated by B cells and specialized macrophages. The B cells in the marginal zone, known as marginal zone B cells, are functionally distinct from follicular B cells and have a limited repertoire of antigen specificities. The architecture of the white pulp is more complex in humans than in mice, with both inner and outer marginal zones and a perifollicular zone. Antigens in the blood are delivered into the marginal sinus by circulating DCs or are sampled by the macrophages in the marginal zone.

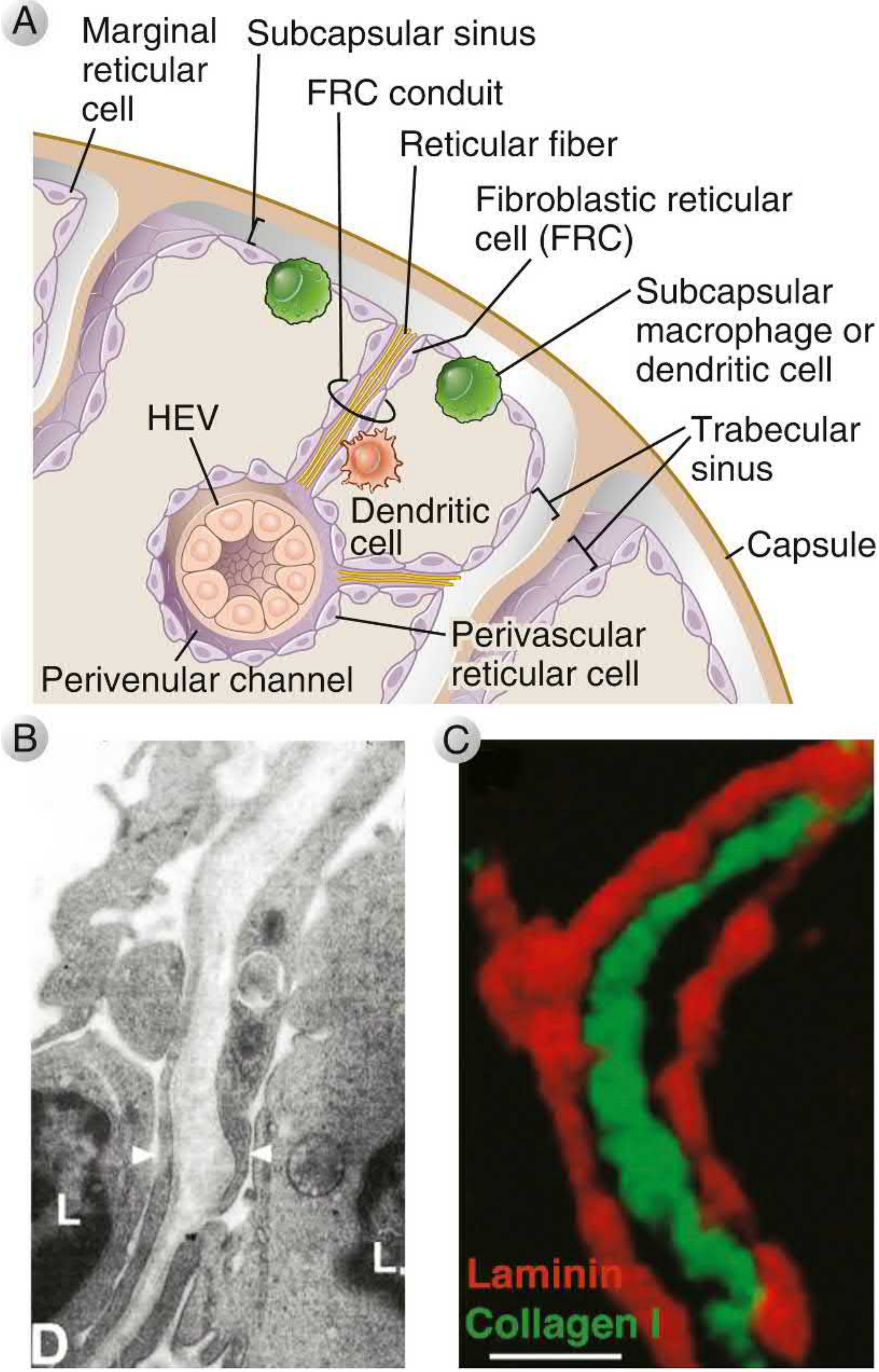

A
Marginal reticular cell
Subcapsular sinus
FRC conduit
Reticular fiber
Fibroblastic reticular cell (FRC)
Subcapsular macrophage or dendritic cell
HEV
Trabecular sinus
Dendritic cell
Capsule
Perivascular reticular cell
Perivenular channel
B
L
L
D
C
Laminin
Collagen I

FIGURE 2.18 Microanatomy of the lymph node cortex. **A,** Schematic of the microanatomy of a lymph node showing locations of fibroreticular cells (FRCs) and depicting the route of lymph drainage from the subcapsular sinus, through FRC conduits, to the perivenular channel around the HEV. **B,** Transmission electron micrograph of an FRC conduit surrounded by fibroblast reticular cells *(arrowheads)* and adjacent lymphocytes *(L)*. **C,** Immunofluorescent stain of an FRC conduit formed of the basement membrane protein laminin *(red)* and collagen fibrils *(green)*. *HEV,* High endothelial venule.

From Gretz JE, Norbury CC, Anderson AO, Proudfoot AEI, Shaw S. Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *J Exp Med.* 2000;192:1425–1439; and Sixt M, Nobuo K, Selg M, Samson T, Roos G, Reinhardt DP, et al. The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity.* 2005;22:19–29. Copyright © 2005 by Elsevier Inc.

The anatomic arrangements of the APCs, B cells, and T cells in the splenic white pulp promote the interactions required for the efficient development of humoral immune responses, as we will discuss in Chapter 12. The segregation of T lymphocytes in the periarteriolar lymphoid sheaths and B cells in follicles and marginal zones is dependent on the production of different cytokines and chemokines by the stromal cells in these different areas, analogous to the case for lymph nodes. As in lymph nodes, the chemokine CXCL13 and its receptor CXCR5 are required for B cell migration into the follicles, and CCL19 and CCL21 and their receptor CCR7 are required for naive T cell migration into the periarteriolar sheath. The production of these chemokines by nonlymphoid stromal cells, such as FRCs, is stimulated by the cytokine lymphotoxin.

Cutaneous and Mucosal Immune Systems

All major epithelial barriers of the body, including the skin, gastrointestinal mucosa, and bronchial mucosa, have their own system of lymph nodes, nonencapsulated lymphoid structures, and diffusely distributed immune cells, which work in coordinated ways to provide specialized immune responses against the pathogens that enter at those barriers. The skin-associated immune system has evolved to respond to a wide variety of environmental microbes. The components of the immune systems associated with the gastrointestinal and bronchial mucosa are called the mucosa-associated lymphoid tissue (MALT) and are involved in immune responses to ingested and inhaled antigens and microbes. The skin and MALT contain a large proportion of the cells of the innate and adaptive immune systems. An important feature of these epithelial tissues is that they are densely populated with commensal microbes, some of which are essential for normal physiology. The immune system in these tissues has evolved to not eliminate the commensals. We will discuss the special features of these epithelial barrier immune systems in Chapter 14.

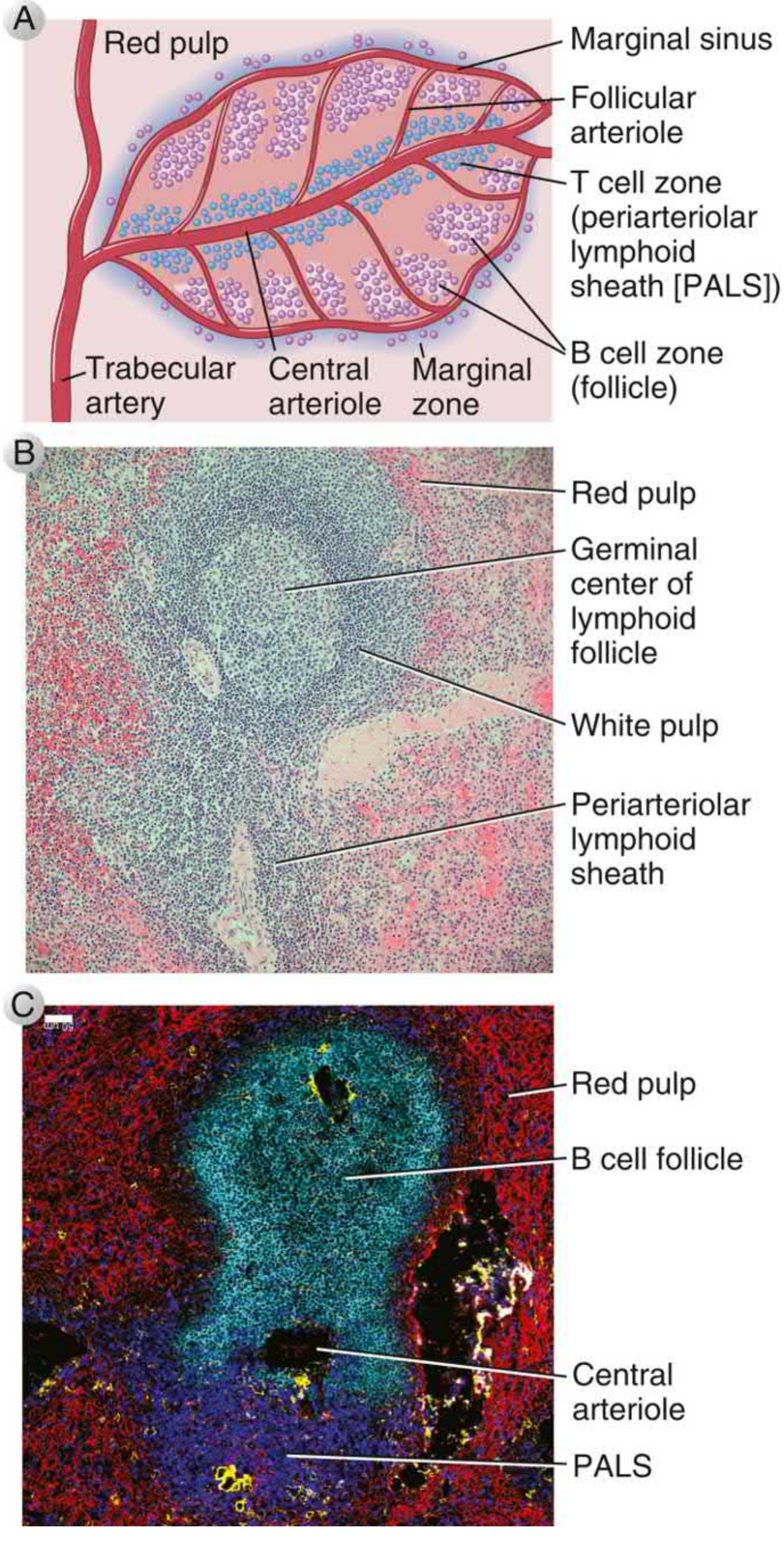

A
Red pulp
Marginal sinus
Follicular arteriole
T cell zone (periarteriolar lymphoid sheath [PALS])
B cell zone (follicle)
Trabecular artery
Central arteriole
Marginal zone
B
Red pulp
Germinal center of lymphoid follicle
White pulp
Periarteriolar lymphoid sheath
C
Red pulp
B cell follicle
Central arteriole
PALS

FIGURE 2.19 Morphology of the spleen. **A,** Schematic diagram of the spleen illustrating T cell and B cell zones, which make up the white pulp. **B,** Photomicrograph of a section of human spleen showing a periarteriolar lymphoid sheath and a lymphoid follicle with a germinal center. Surrounding these areas is the red pulp, rich in vascular sinusoids. **C,** Immunofluorescence stain of white pulp in human spleen, demonstrating B cells, *light blue,* in a follicle and T cells, stained *dark blue,* in the periarteriolar lymphoid sheath.

B, Courtesy of Kaushik Sridhar and Robert Ohgami, MD, PhD, Department of Pathology, University of California San Francisco, San Fracisco, California. *C,* Courtesy of Andrea Radtke, PhD, laboratory of Ronald N. Germain, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Summary

- The anatomic organization of the cells and tissues of the immune system is of critical importance for the generation of effective innate and adaptive immune responses. This organization permits the rapid delivery of innate immune cells, including neutrophils and monocytes, to sites of infection and permits a small number of lymphocytes specific for any antigen to locate and respond effectively to that antigen regardless of where in the body the antigen is introduced.
- The cells that perform the majority of effector functions of innate and adaptive immunity are phagocytes (including neutrophils and macrophages), mast cells, basophils, eosinophils, dendritic cells, innate lymphoid cells (ILCs), and natural killer (NK) cells, and lymphocytes.

- Many surface molecules are differentially expressed on distinct types and subsets of immune cells, and these are named according to the CD nomenclature.
- Neutrophils, the most abundant blood leukocyte with a distinctive multilobed segmented nucleus and abundant cytoplasmic lysosomal granules, are rapidly recruited to sites of infection and tissue injury, where they perform phagocytic and microbial killing functions.
- Tissue resident–macrophages are sentinel cells that detect microbes and alert the immune system and may perform other specialized functions in different tissues, such as lung, spleen, and liver.
- Monocytes are circulating phagocytes that are recruited to sites of tissue infection and injury, where they rapidly differentiate into macrophages that ingest and kill microbes and dead host cells and secrete cytokines and chemokines that promote the recruitment of leukocytes from the blood and initiate the repair of damaged tissues.
- Dendritic cells (DCs) are bone marrow–derived cells with extended cytoplasmic processes that are present in most tissues of the body and function as innate sentinel cells and as antigen-presenting cells (APCs) uniquely capable of activating naive T lymphocytes. There are different subsets of DCs with different functions in innate and adaptive immunity.
- Innate lymphoid cells (ILCs) are cytokine-producing cells of the innate immune system with a lymphocyte-like morphology. They perform functions similar to those of $CD4^+$ or $CD8^+$ effector T cells. ILCs, which include NK cells, do not express highly diverse, clonally distributed antigen receptors.
- B and T lymphocytes express highly diverse and specific antigen receptors and are the cells responsible for the specificity and memory of adaptive immune responses.

- Both B and T lymphocytes arise from a common precursor in the bone marrow. B cell development proceeds in the bone marrow, whereas T cell precursors migrate to and mature in the thymus. After maturing, B and T cells leave the bone marrow or thymus, enter the circulation, and populate peripheral lymphoid organs.
- Naive B and T cells are mature lymphocytes that have not been previously stimulated by antigen. When they encounter antigen, they proliferate and differentiate into effector lymphocytes that have functions in protective immune responses. Effector B lymphocytes are antibody-secreting plasma cells. Effector T cells include cytokine-secreting $CD4^+$ helper T cells and $CD8^+$ CTLs.
- Some of the progeny of antigen-activated B and T lymphocytes differentiate into memory cells that survive for long periods in a quiescent state. These memory cells are responsible for the rapid and enhanced responses to subsequent exposures to antigen.
- The organs of the immune system may be divided into the primary, or generative, lymphoid organs (bone marrow and thymus), where lymphocytes mature, and the secondary, or peripheral, lymphoid organs (lymph nodes, spleen, and the mucosal and cutaneous immune systems), where lymphocytes are activated by antigens.
- Bone marrow contains the stem cells for all blood cells, including lymphocytes, and is the site of maturation of all of these cell types except T cells, which mature in the thymus.
- Extracellular fluid (lymph) is constantly drained from tissues through lymphatics into lymph nodes and eventually into the blood. Microbial antigens are carried in soluble form and within DCs in the lymph to lymph nodes, where they are recognized by lymphocytes.
- Lymph nodes are encapsulated secondary lymphoid organs located throughout the body along lymphatics, where naive

B and T cells respond to antigens that are collected by the lymph from peripheral tissues.

- The spleen is an encapsulated organ in the abdominal cavity where senescent or opsonized blood cells are removed from the circulation, and in which lymphocytes respond to blood borne antigens.
- Lymph nodes and the white pulp of the spleen are organized into B cell zones (the follicles) and T cell zones. The T cell areas are also the sites of residence of mature DCs, which are APCs specialized for the activation of naive T cells.
- Fibroblastic reticular cells (FRCs) are specialized myofibroblasts found in secondary lymphoid organs, which are essential for the structural organization and function of these organs. FRCs produce chemokines and form FRC-conduits, both of which are required for the directed movement of lymphocytes and DCs within the organs, and the separation of B and T cells. Follicular dendritic cells are related to FRCs and are required for B cell follicle structure and function.
- The development of secondary lymphoid tissues depends on cytokines and lymphoid tissue inducer cells.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

Cells of the Immune System

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∗Cooper M.D, Raymond D.A, Peterson R.D, et al. The functions of the thymus system and the bursa system in the chicken. *J Exp Med* . 1966;123:75–106Miller, 1961 Miller J.F. Immunological function of the thymus. *Lancet* . 1961;2:748–749 *(The discoveries that T and B cell lineages are respectively responsible for cell-mediated and humoral immunity.)*.

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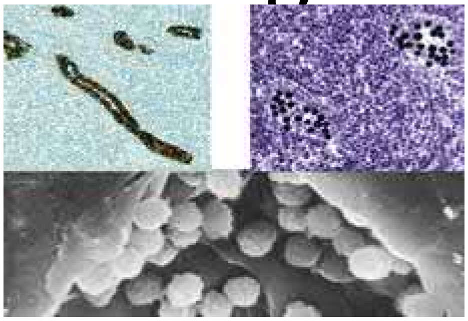
Chapter 3: Leukocyte Circulation and Migration Into Tissues



Overview of Leukocyte Migration,

Adhesion Molecules on Leukocytes and Endothelial Cells Involved in Leukocyte Recruitment,
    Selectins and Selectin Ligands,
    Integrins and Integrin Ligands,

Chemokines and Chemokine Receptors,
    Chemokine Structure, Production, and Receptors,
    Functions of Chemokines,
    Other Chemoattractants and Receptors,

Leukocyte-Endothelial Interactions and Leukocyte Recruitment into Tissues,

Migration of Neutrophils and Monocytes to Sites of Infection or Tissue Injury,

Migration and Recirculation of T Lymphocytes,
  - Recirculation of Naive T Lymphocytes Between Blood and Secondary Lymphoid Organs,
  - Recirculation of T Cells Through Other Lymphoid Tissues,
  - Migration of Effector T Lymphocytes to Sites of Infection,
  - Memory T Cell Migration,

Migration of B Lymphocytes,
  - Recirculation of Naive Follicular B Cells,
  - Migration of Antibody Secreting Plasmablasts and Memory B Cells,

Summary,

A unique property of the immune system that distinguishes it from all other physiological systems in the body is the constant and highly regulated movement of its major cellular components through the blood, into tissues, and often back into the blood again. This movement accomplishes three main functions (Fig. 3.1):

- Delivery of leukocytes of myeloid lineage (mainly neutrophils and monocytes) from the circulation into tissue sites of infection or injury, where the cells perform their protective functions of eliminating infectious pathogens, clearing dead tissues, and repairing the damage.
- Delivery of lymphocytes from their sites of maturation (bone marrow or thymus) to secondary lymphoid organs, where the cells recognize antigens, proliferate, and differentiate into effector and memory lymphocytes.
- Delivery of effector lymphocytes from the secondary lymphoid organs in which they were produced to sites of infection in any tissue, where they perform their protective functions.

Because of the ability of immune cells to disseminate throughout the body, an immune response may be initiated at one site but may be active at distant locations. In other words, immunity is both local and systemic.

The migration of a leukocyte out of the blood and into a particular tissue, or to a site of an infection or injury, is often called leukocyte **homing,** and the general process of leukocyte movement from blood into tissues is called **migration** or **recruitment.** The ability of lymphocytes to repeatedly home to secondary lymphoid organs, reside there transiently, and return to the blood is called **recirculation.** The recruitment of leukocytes and plasma proteins from the blood to sites of infection and tissue injury is a major part of the process of **inflammation.** Inflammation is triggered by recognition of microbes and injured or dead cells in innate immune responses and is refined and prolonged during adaptive immune responses. The inflammatory response delivers the cells and molecules of host defense to the sites where offending agents need to be combated. The same process is responsible for causing tissue damage and underlies many important diseases. We will return to inflammation in the context of innate immunity in Chapter 4 and in the discussion of inflammatory diseases in Chapter 19.

Overview of Leukocyte Migration

Leukocyte homing and recruitment to different tissues are governed by some general principles.

- Naive lymphocytes continuously migrate mainly into secondary lymphoid organs, whereas lymphocytes that have been previously activated by antigen (e.g., effector lymphocytes), as well as myeloid leukocytes, preferentially home into tissues where there is infection or tissue injury. Memory lymphocytes migrate into lymphoid organs, mucosal tissues, skin, and other tissues.

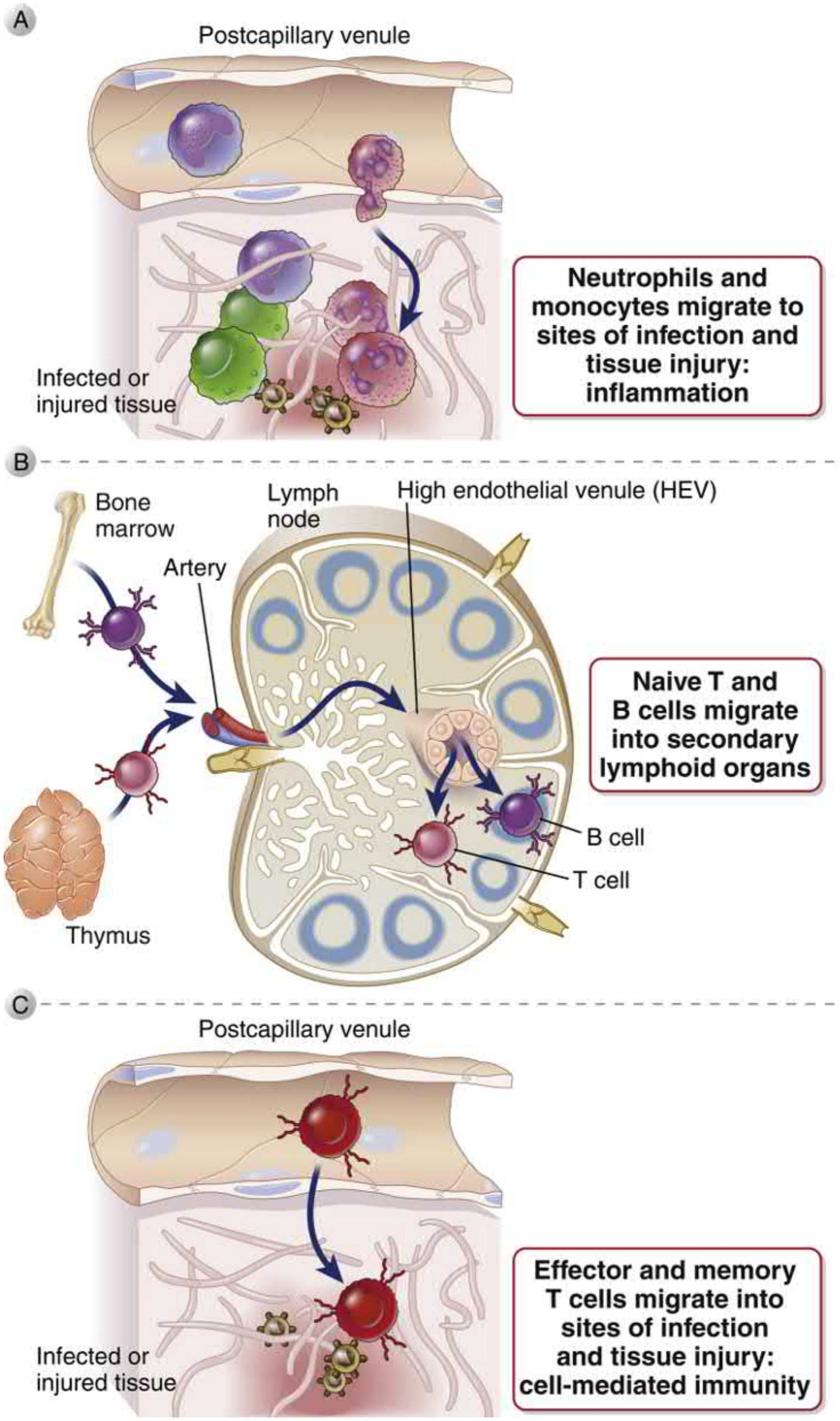
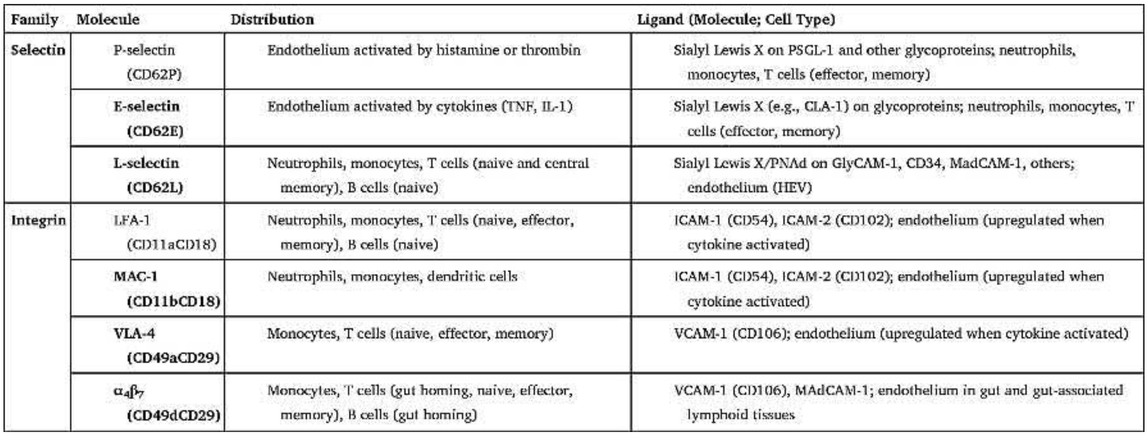

A
Postcapillary venule
Infected or injured tissue
Neutrophils and monocytes migrate to sites of infection and tissue injury: inflammation
B
Bone marrow
Lymph node
High endothelial venule (HEV)
Artery
Naive T and B cells migrate into secondary lymphoid organs
B cell
T cell
Thymus
C
Postcapillary venule
Infected or injured tissue
Effector and memory T cells migrate into sites of infection and tissue injury: cell-mediated immunity

FIGURE 3.1 The main functions served by leukocyte migration from blood into tissues.

A, Neutrophils and monocytes that arise in the bone marrow, circulate in the blood, and are recruited into tissue sites of infection or injury, where they eliminate infectious pathogens, clear dead tissues, and repair the damage. **B,** Naive lymphocytes that arise in bone marrow or thymus home to secondary lymphoid organs, such as lymph nodes (or spleen, not shown), where they become activated by antigens and differentiate into effector lymphocytes. **C,** Effector lymphocytes that develop in secondary lymphoid organs migrate into tissue sites of infection, where they participate in microbial defense. Memory lymphocytes (not shown) migrate between among, secondary lymphoid organs, and normal or infected tissues.

TABLE 3.1



Family	Molecule	Distribution	Ligand (Molecule; Cell Type)
Selectin	P-selectin (CD62P)	Endothelium activated by histamine or thrombin	Sialyl Lewis X on PSGL-1 and other glycoproteins; neutrophils, monocytes, T cells (effector, memory)
	E-selectin (CD62E)	Endothelium activated by cytokines (TNF, IL-1)	Sialyl Lewis X (e.g., CLA-1) on glycoproteins; neutrophils, monocytes, T cells (effector, memory)
	L-selectin (CD62L)	Neutrophils, monocytes, T cells (naive and central memory), B cells (naive)	Sialyl Lewis X/PNAd on GlyCAM-1, CD34, MadCAM-1, others; endothelium (HEV)
Integrin	LFA-1 (CD11aCD18)	Neutrophils, monocytes, T cells (naive, effector, memory), B cells (naive)	ICAM-1 (CD54), ICAM-2 (CD102); endothelium (upregulated when cytokine activated)
	MAC-1 (CD11bCD18)	Neutrophils, monocytes, dendritic cells	ICAM-1 (CD54), ICAM-2 (CD102); endothelium (upregulated when cytokine activated)
	VLA-4 (CD49aCD29)	Monocytes, T cells (naive, effector, memory)	VCAM-1 (CD106); endothelium (upregulated when cytokine activated)
	$\alpha_4\beta_7$ **(CD49dCD29)**	Monocytes, T cells (gut homing, naive, effector, memory), B cells (gut homing)	VCAM-1 (CD106), MAdCAM-1; endothelium in gut and gut-associated lymphoid tissues

CLA-1, Cutaneous lymphocyte antigen 1; *GlyCAM-1,* glycan-bearing cell adhesion molecule 1; *HEV,* high endothelial venule; *ICAM-1,* intracellular adhesion molecule 1; *IL-1,* interleukin-1; *LFA-1,* leukocyte function–associated antigen 1; *MAdCAM-1,* mucosal addressin cell adhesion molecule 1; *PNAd,* peripheral node addressin; *PSGL-1,* P-selectin glycoprotein ligand 1; *TNF,* tumor necrosis factor; *VCAM-1,* vascular cell adhesion molecule 1; *VLA-4,* very late antigen 4.

- Leukocyte homing and recruitment require the adhesion of leukocytes to the endothelial lining of postcapillary venules, a process that involves molecules on the surfaces of both the leukocytes (adhesion molecules and chemokine receptors) and endothelial cells (adhesion molecules and chemokines).
- Endothelial cells at sites of infection and tissue injury are activated by cytokines secreted by sentinel cells in the tissues (including dendritic cells [DCs], macrophages, and mast cells), which induce increased expression of adhesion molecules and chemokines. The consequence is increased adhesiveness of the endothelial cells for circulating myeloid leukocytes and previously activated lymphocytes.
- Because microbes and necrotic tissues stimulate the expression of molecules that mediate leukocyte-endothelial adhesion at the sites of infection or injury, effector leukocytes migrate through endothelium mainly when and where they are needed.

The basic process of leukocyte migration into tissues is the same for the homing of different types of leukocytes (neutrophils, monocytes, and naive and effector lymphocytes) to different types of tissues (secondary lymphoid organs, infected tissues), although the specific chemokines and adhesion molecules vary in ways that result in distinct patterns of migration for different cell types. Before describing the process, we will discuss the properties and functions of the adhesion molecules and chemokines that are involved in leukocyte recruitment.

Adhesion Molecules on Leukocytes and Endothelial Cells Involved in Leukocyte Recruitment

Adhesion of circulating leukocytes to vascular endothelial cells is mediated by two classes of molecules, called selectins and integrins,

and their ligands. The expression of these molecules varies among different types of leukocytes and in blood vessels at different locations.

Selectins and Selectin Ligands

Selectins are plasma membrane carbohydrate-binding adhesion molecules that mediate an initial step of low-affinity adhesion of circulating leukocytes to endothelial cells lining postcapillary venules (Table 3.1) **.** The extracellular domains of selectins are similar to C-type lectins, so called because they bind carbohydrate structures (lectins are carbohydrate-binding proteins) in a calcium-dependent manner. Selectins and their ligands are expressed on leukocytes and endothelial cells. Importantly, the expression of selectins and their ligands is increased by exposure to cytokines produced at sites of tissue injury and infection, and this is one mechanism by which leukocytes are recruited to sites of inflammation.

Endothelial cells express two types of selectins, called **P-selectin** (CD62P) and **E-selectin** (CD62E). P-selectin, so named because it was first found in platelets, is stored in cytoplasmic granules of endothelial cells and is rapidly redistributed to the luminal surface in response to histamine from mast cells and thrombin generated during blood coagulation. E-selectin is synthesized and expressed on the endothelial cell surface within 1 to 2 hours in response to the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF), which are produced by tissue sentinel cells (DCs and macrophages) in response to infection. Microbial products such as lipopolysaccharide (LPS) also stimulate E-selectin expression on endothelial cells. We will describe IL-1, TNF, and LPS in our discussion of inflammation in Chapter 4.

The ligands on leukocytes that bind to E-selectin and P-selectin on endothelial cells are complex sialylated carbohydrates related to the Lewis X or Lewis A family of blood group molecules. These chemical structures are present on various surface glycoproteins of granulocytes, monocytes, and some previously activated effector and memory T cells. The best defined of these is the tetrasaccharide sialyl Lewis X. (The structure was originally identified as an antigen on red blood cells in studies of genetically related people with the family name Lewis). A leukocyte membrane glycoprotein called P-selectin glycoprotein ligand 1 (PSGL-1) is post-translationally modified to display sialyl Lewis X, which serves as the major carbohydrate ligand for P-selectin. Several different molecules may display the carbohydrate ligand for E-selectin, including the glycoproteins PSGL-1 and E-selectin ligand-1 and some glycolipids.

A third selectin, called **L-selectin** (CD62L), is expressed on leukocytes and not on endothelial cells. The ligands for L-selectin are sialomucins on endothelial cells, whose expression may be increased by cytokine activation of the cells. A major recognition determinant that L-selectin binds to on these sialomucins is sialyl 6-sulfo Lewis X. The expression of the ligand on endothelial cells is increased by IL-1, TNF, and other inflammatory cytokines, so L-selectin on neutrophils promotes the adhesion of these cells to endothelial cells at sites of inflammation. In adaptive immunity, L-selectin is required for naive T and B lymphocyte homing into lymph nodes through specialized blood vessels called **high endothelial venules (HEVs).** The sialomucin ligands on HEVs that bind to L-selectin on naive lymphocytes are collectively called peripheral node addressin (PNAd). Leukocytes express L-selectin and the carbohydrate ligands for P-selectin and E-selectin at the tips of their microvilli, facilitating interactions with molecules on the endothelial cell surface.

Integrins and Integrin Ligands

Integrins are cell surface proteins that mediate adhesion of cells to other cells or to extracellular matrix, through specific binding interactions with various ligands. There are more than 30 different integrins, all of which are heterodimers containing one of more than 15 types of α chains and one of seven types of β chains. The extracellular globular heads of both chains contribute to ligand binding. The cytoplasmic domains of the integrins interact with cytoskeletal components (including vinculin, talin, actin, α-actinin, and tropomyosin). The name integrin for this family of proteins derives from the idea that these proteins integrate signals triggered by extracellular ligands with cytoskeleton-dependent motility, shape change, and phagocytic responses. Although we will focus on the leukocyte adhesive functions of integrins, these molecules have many other biological functions, including adhesion, proliferation and differentiation of epithelial cells, and platelet adhesion and aggregation during blood coagulation. Inflammatory cytokines and other signals increase endothelial expression of integrin ligands and binding affinity of leukocyte integrins, thus promoting leukocyte adhesion to endothelium at sites of inflammation.

In the immune system, two important integrins that are expressed on myeloid cells and lymphocytes are leukocyte function–associated antigen 1 **(LFA-1),** more precisely named $\alpha_L\beta_2$ or CD11aCD18, and very late antigen 4 **(VLA-4),** also named $\alpha_4\beta_1$ or CD49dCD29 (see Table 3.1). One important ligand for LFA-1 is intercellular adhesion molecule 1 (**ICAM-1,** CD54), a membrane glycoprotein expressed on cytokine-activated endothelial cells and on a variety of other cell types, including lymphocytes, DCs, macrophages, fibroblasts, and most epithelial cells. The extracellular portion of ICAM-1 is composed of globular domains, called immunoglobulin (Ig) domains, which share sequence homology and structural features with domains found in Ig molecules. Many proteins in the immune system contain Ig domains and belong to the Ig superfamily (see Chapter 5). LFA-1 binding to ICAM-1 is important for leukocyte-endothelial interactions (discussed later) and T cell interactions with antigen-presenting cells (APCs) (see Chapter 9). Two other Ig superfamily ligands for LFA-1 are ICAM-2, which is expressed on endothelial cells, and ICAM-3, which is expressed on lymphocytes. VLA-4 binds to vascular cell adhesion molecule 1 (**VCAM-1,** CD106), an Ig superfamily protein expressed on cytokine-activated endothelial cells in some tissues. Other integrins also play roles in innate and adaptive immune responses. For example, **MAC-1** ($\alpha_M\beta_2$, CD11bCD18, or complement receptor 3 [CR3]) on circulating monocytes binds to ICAM-1 and mediates adhesion to endothelium. MAC-1 also functions as a complement receptor, as does another integrin of the β2 family known as CD11cCD18 ($\alpha_X\beta_2$, complement receptor 4 [CR4]); both bind particles opsonized with a product of complement activation called the inactivated C3b (iC3b) fragment (discussed in Chapters 4 and 13), and thereby enhance phagocytosis of microbes. CD11cCD18 is expressed mainly on DCs but also on other myeloid cells and activated B cells. It helps DCs ingest apoptotic cells. The integrin $\alpha_4\beta_7$ is expressed on lymphocytes that home to intestinal mucosa and binds to an endothelial protein called mucosal addressin cell adhesion molecule 1 (MAdCAM-1). $\alpha_E\beta_7$ (CD103) is an integrin that binds to an epithelial adhesion molecule called E-cadherin. $\alpha_E\beta_7$ is expressed on subsets of T cells and DCs that are found within epithelial layers of mucosa.

Integrins rapidly increase affinity for their ligands in response to intracellular signals, which are induced in all leukocytes by chemokine binding to chemokine receptors and in T cells by antigen binding to antigen receptors. Chemokine receptor and antigen receptor engagement trigger signaling pathways (described in more detail in Chapter 7) that lead to the association of RAP family small GTPase proteins and cytoskeleton-interacting proteins with the cytoplasmic tails of the integrin proteins. This results in conformational changes in the extracellular domains of the integrins that lead to increased affinity (Fig. 3.2). In the low-affinity state, the stalks of the extracellular domains of each integrin subunit are bent over, and the ligand-binding globular heads are close to the plasma membrane. In response to alterations in the cytoplasmic tail, the stalks extend, bringing the globular heads away from the membrane to a position where they more effectively interact with their ligands. The process by which intracellular signals, generated in response to chemokines or antigen, alter the binding functions of the extracellular domain of integrins is called inside-out signaling.

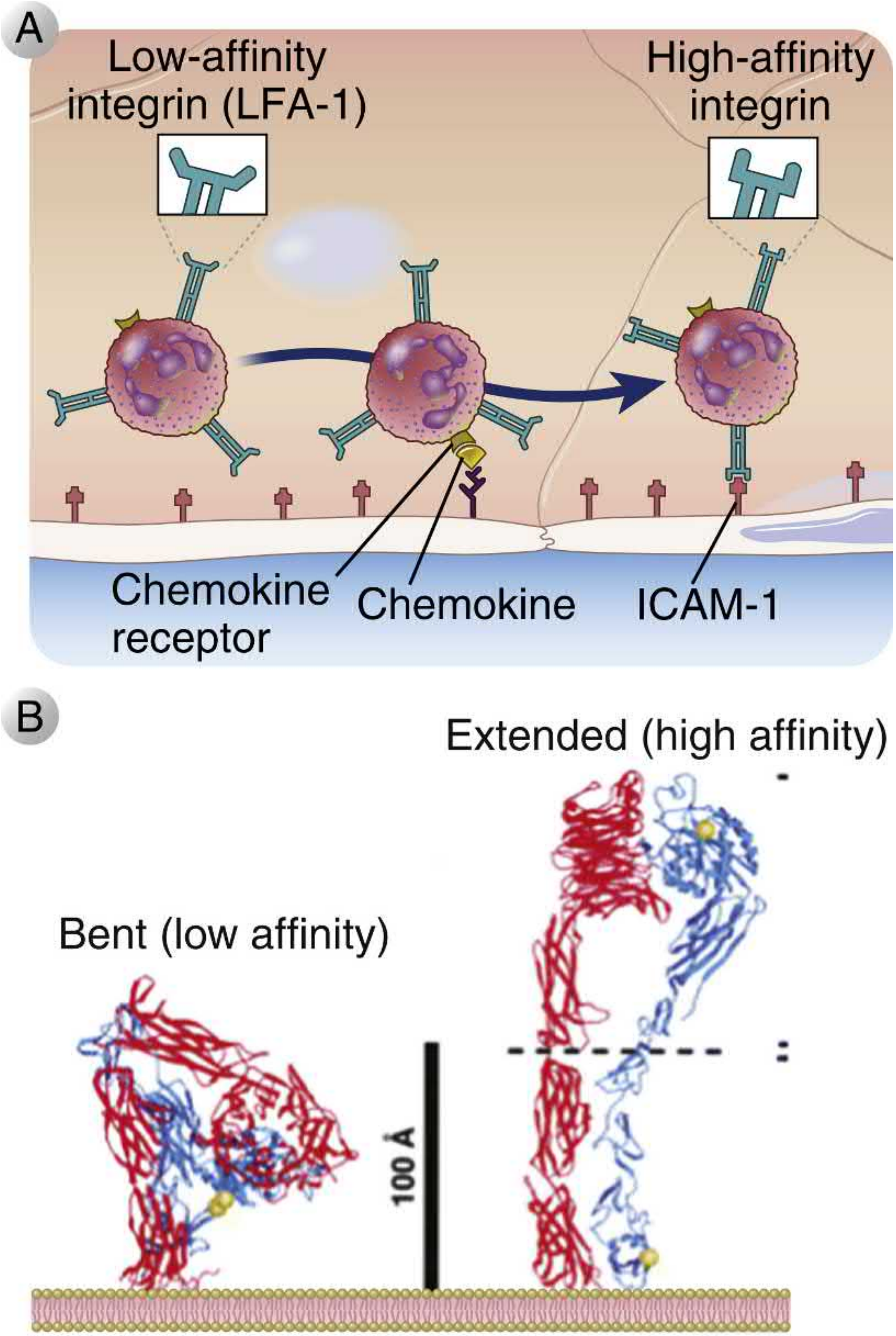

A
Low-affinity integrin (LFA-1)
High-affinity integrin
Chemokine receptor
Chemokine
ICAM-1
B
Extended (high affinity)
Bent (low affinity)
100 Å

FIGURE 3.2 Integrin activation. **A,** The integrins on blood leukocytes are normally in a low-affinity state. If a leukocyte comes close to endothelial

cells, such as when selectin-dependent rolling of leukocytes occurs, then chemokines displayed on the endothelial surface can bind chemokine receptors on the leukocyte. Chemokine receptor signaling then occurs, which activates the leukocyte integrins, increasing their affinity for their ligands on the endothelial cells. **B,** Ribbon diagrams are shown of bent and extended conformations of a leukocyte integrin, corresponding to low- and high-affinity states, respectively. *ICAM-1,* Intercellular adhesion molecule 1.

B, From Takagi J, Springer TA. Integrin activation and structural rearrangement.*Immunol Rev*. 2002;186:141–163.

Chemokines also induce clustering of integrins on leukocyte surfaces. This results in higher local concentration of integrins at the sites of interaction with endothelial cells, where the chemokines are displayed, leading to increased overall strength (avidity) of integrin-mediated leukocyte binding to the endothelium.

Chemokines and Chemokine Receptors

Chemokines are a large family of structurally homologous cytokines that stimulate leukocyte movement and regulate the migration of leukocytes from the blood to tissues. Chemokines stimulate movement of leukocytes along the concentration gradient of the secreted proteins toward their source, a process called chemotaxis (or chemoattraction). The name chemokine is a contraction of chemotactic cytokine. We referred to the role of chemokines in the organization of lymphoid tissues in Chapter 2, and now we will describe the general properties of this family of cytokines and their multiple functions in innate and adaptive immunity. Table 3.2 summarizes the major features of selected chemokines and their receptors.

Chemokine Structure, Production, and Receptors

Most chemokines are 8- to 10-kD polypeptides that contain two internal disulfide loops and four cysteine residues that are responsible for the tertiary structure. There are 47 human chemokines, which are classified into four families on the basis of the number and location of two of the conserved cysteines. The two major families are the CC (also called β) chemokines, in which the two defining cysteine residues are adjacent, and the CXC (or *α*) chemokines, in which these residues are separated by one amino acid. In both mice and humans, the genes encoding most of the CC or CXC chemokines are found in distinct clusters on different chromosomes. A few additional chemokines have either a single cysteine (C family) or two cysteines separated by three amino acids (CX3C). There are two structural variants of CXC chemokines, some that have the amino acid sequence glutamic acid–leucine-arginine (called ELR motifs) just before the first cysteine of the CXC motif and others without the ELR motif. CXC chemokines with ELR motifs support neutrophil migration. The other CXC chemokines and the CC chemokines act on monocytes, lymphocytes, and other leukocytes. Chemokines were originally named on the basis of how they were identified and what responses they triggered, but a standard nomenclature has been adopted and coordinated with names for the receptors the chemokines bind to (see Table 3.2). The CC chemokines are named CCL1 through CCL28, and the CXC chemokines are named CXCL1 through CXCL17.

Chemokines bind tightly to glycosaminoglycans with sulfated N-acetyllactosamine disaccharide repeat units, and there is specificity for which chemokines bind depending on the length of the chain of repeats and the pattern of sulfation. These glycosaminoglycans on the surface of endothelial cells or in extravascular matrices display the bound chemokines to chemokine receptors expressed on migrating cells.

The chemokines of the CC and CXC subfamilies are produced by leukocytes and by several types of tissue cells, such as endothelial cells, epithelial cells, resident macrophages, fibroblasts, and other stromal cells. In many of these cells, secretion of chemokines is induced by recognition of microbes through various cellular receptors of the innate immune system, discussed in Chapter 4. In addition, inflammatory cytokines, including TNF, IL-1, and IL-17, induce chemokine production. Several CC chemokines are also produced by activated T cells, providing a link between adaptive immunity and recruitment of inflammatory leukocytes.

TABLE 3.2

Chemokine	Original Name	Chemokine Receptor	Major Function
CC Chemokines			
CCL2	MCP-1	CCR2	Mixed leukocyte recruitment
CCL3	MIP-1α	CCR1, CCR5	Mixed leukocyte recruitment
CCL4	MIP-1β	CCR5	T cell, dendritic cell, monocyte, and NK recruitment; HIV coreceptor
CCL5	RANTES	CCR1, CCR3, CCR5	Mixed leukocyte recruitment
CCL11	Eotaxin	CCR3	Eosinophil, basophil, and Th2 recruitment
CCL17	TARC	CCR4	T cell recruitment
CCL19	MIP- β/ELC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL21	SLC	CCR7	T cell and dendritic cell migration into parafollicular zones of lymph nodes
CCL22	MDC	CCR4	NK cell, T cell recruitment
CCL25	TECK	CCR9	Lymphocyte recruitment into intestine
CCL27	CTACK	CCR10	T cell recruitment into skin
CXC Chemokines			
CXCL1	GROα	CXCR2	Neutrophil recruitment
CXCL8	IL-8	CXCR1, CXCR-2	Neutrophil recruitment
CXCL9	Mig	CXCR3	Effector T cell recruitment
CXCL10	IP-10	CXCR3	Effector T cell recruitment
CXCL12	SDF1	CXCR4	B and T cell migration into lymph nodes; plasma cell migration into bone marrow
CXCL13	BCA-1	CXCR5	B cell migration into lymph nodes and into follicles; T follicular helper cell migration into follicles
C Chemokines			
XCL1	Lymphotactin	XCR1	T cell and NK cell recruitment
CX3C Chemokines			
CX3CL1	Fractalkine	CX3CR1	T cell, NK cell, and monocyte recruitment

CTL, Cytotoxic T lymphocyte; *IL,* Interleukin; *NK,* natural killer cells.

The receptors for chemokines belong to the seven-transmembrane, guanosine triphosphate–binding (G) protein–coupled receptor (GPCR) superfamily. These receptors initiate intracellular responses through associated trimeric G proteins. All chemokine receptors mediating immune cell migration share an amino acid sequence motif (DRYLAIV) at the end of the third transmembrane domain that is required for interaction with G proteins. The G proteins stimulate signaling events that result in cytoskeletal changes and polymerization of actin and myosin filaments, resulting in increased cell motility. As previously discussed, these signals also increase the affinity of the integrins for their ligands.

Different combinations of chemokine receptors are expressed on different types of leukocytes, resulting in distinct patterns of migration of these cells. There are 10 different receptors for CC chemokines (called CCR1 through CCR10), seven for CXC chemokines (called CXCR1 through CXCR6 and CXCR8), one for the C chemokine (called XCR1), and one for CX3CL1 (called CX3CR1) (see Table 3.2). Chemokine receptors are expressed on all leukocytes, with the greatest number and diversity seen on T cells. The receptors exhibit overlapping specificity for chemokines within each family, and the pattern of cellular expression of the receptors determines which cell types respond to which chemokines. Certain chemokine receptors, notably CCR5 and CXCR4, act as coreceptors for entry of human immunodeficiency virus (HIV) into cells (see Chapter 21).

A distinct set of chemokine receptors, called atypical chemokine receptors (ACKRs), does not engage heterodimeric G-protein signaling pathways that activate leukocytes but rather is involved in inhibiting or terminating chemokine responses in cells. There are four human ACKRs, expressed on many different cell types, that together bind most of the inflammatory chemokines with high affinity. One particular ACKR, ACKR1 or DARC, is found on erythrocytes and on the specialized endothelium of postcapillary venules (the site of leukocyte migration from the blood into tissues). ACKRs signal through a pathway dependent on G-protein regulatory proteins called β-arrestins, which bind to chemokine receptors with attached chemokines and simulate the internalization and degradation of these receptors.

Functions of Chemokines

Some chemokines are produced by cells in response to external stimuli and are involved in inflammatory reactions. Other chemokines are produced constitutively in tissues and maintain the distribution of cells in these tissues, such as localization of T and B cells in lymphoid organs.

- ***In inflammatory reactions, chemokines serve to recruit circulating leukocytes from blood vessels into extravascular sites.*** Different groups of chemokines bind to chemokine receptors expressed on different cells and, in coordination with the types of adhesion molecules expressed, control the nature of the inflammatory infiltrate. Chemokines play two roles in inflammation.
  - ***Increased adhesion of leukocytes to endothelium.*** Chemokines produced in the tissues bind to heparan sulfate proteoglycans on endothelial cells that line postcapillary venules. The bound chemokines are displayed in this way to circulating leukocytes that are attached to the endothelial surfaces through adhesion molecule interactions. Endothelial display provides a high local concentration of chemokines, enabling them to bind to chemokine receptors on the leukocytes. Signals from chemokine receptors lead to enhanced integrin affinity, which results in firm adhesion of the leukocyte, a critical step for migration of leukocytes out of blood vessels into extravascular tissue.
  - ***Migration of leukocytes through blood vessels and toward the site of infection or tissue damage.*** Chemokines produced in the extravascular tissues act on leukocytes that have migrated through the endothelium and exited the circulation. The leukocytes migrate toward infected and damaged cells in tissues, where chemokines are produced and are at the highest concentration.

- ***Chemokines are involved in the development of lymphoid organs, and they regulate the traffic of lymphocytes and other leukocytes through different regions of secondary lymphoid organs.*** Because these chemokines are expressed constitutively and maintain normal tissue architecture, they are referred to as homeostatic. We discussed the function of chemokines in the anatomic organization of lymphoid organs in Chapter 2. Some homeostatic chemokines are also induced under inflammatory conditions and contribute to leukocyte migration out of blood vessels into tissues.
- ***Chemokines are required for the migration of DCs from sites of infection into draining lymph nodes.*** DCs are activated by microbes in peripheral tissues, and they then migrate to lymph nodes to inform T lymphocytes of the presence of infection (discussed in Chapter 6). This migration depends on expression of a chemokine receptor, CCR7, which is induced when the DCs encounter microbes, and chemokines produced in lymphatics and lymphoid tissues that bind to CCR7. Naive T cells also express CCR7, and this explains how DCs and naive T cells localize to the same place in lymph nodes, enabling the DCs to present antigen to the T cells.

Other Chemoattractants and Receptors

Several other types of molecules serve similar functions as chemokines in the immune system, promoting leukocyte migration into inflammatory sites and their directed movement in tissues or maintaining spatial separation of lymphocytes in secondary lymphoid organs. The C3a and C5a complement fragments generated by activation of complement (described in Chapters 4 and 13), and the arachidonic acid metabolite leukotriene B4 (LTB4) are important chemoattractants that promote neutrophil and monocyte migration during acute inflammatory responses. Other lipid molecules play critical roles in movement of lymphocytes and DCs in lymph nodes, spleen, and mucosal lymphoid tissues. These include sphingosine 1-phosphate, a lipid required for egress of lymphocytes out of lymphoid organs, which is discussed later in this chapter, and oxysterols involved in organizing lymphoid follicles and directing movement of B cells during germinal center reactions, discussed in Chapter 12. All of these chemoattractant molecules, like chemokines, bind to specific GPCRs expressed on the leukocytes.

Leukocyte-Endothelial Interactions and Leukocyte Recruitment into Tissues

Leukocyte recruitment from the blood into tissues requires adhesion of the leukocytes to the endothelial lining of postcapillary venules and then movement through the endothelium and vessel wall into the extravascular tissue. Leutkocyte recruitment into tissues is a key event in inflammatory reactions. It is a multistep process in which each step is orchestrated by different types of adhesion molecules and chemokines. Studies of the interactions of leukocytes with endothelium in vitro under conditions that mimic flowing blood, and in vivo using intravital microscopic techniques, have established a sequence of events common to migration of most leukocytes into most tissues (Fig. 3.3). The steps in this process are the following:

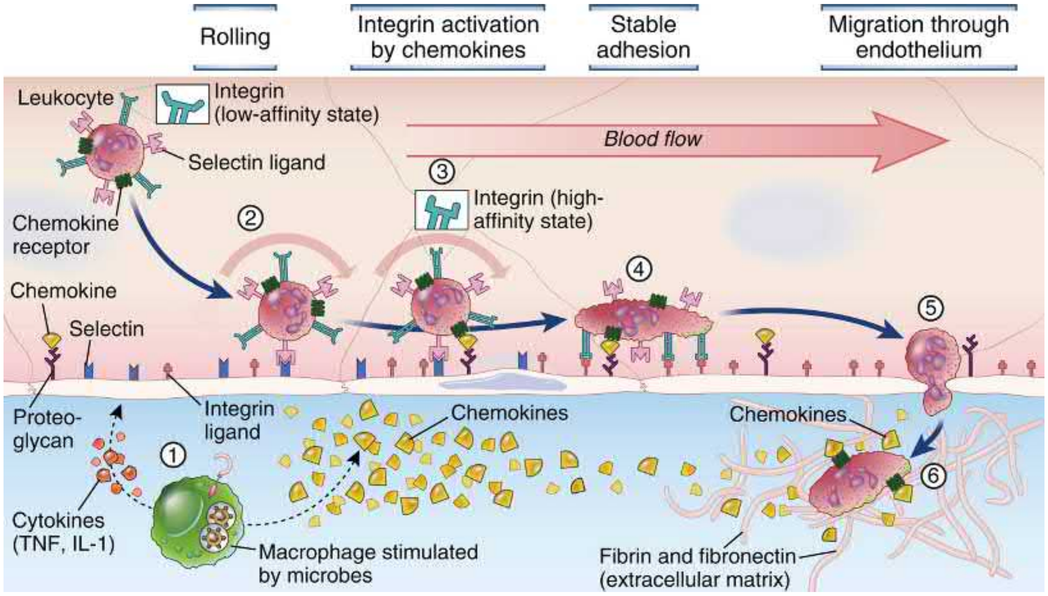

Rolling
Integrin activation by chemokines
Stable adhesion
Migration through endothelium
Leukocyte
Integrin (low-affinity state)
Selectin ligand
Blood flow
③
Integrin (high-affinity state)
Chemokine receptor
②
Chemokine
Selectin
④
⑤
Proteo-glycan
Integrin ligand
Chemokines
Chemokines
①
⑥
Cytokines (TNF, IL-1)
Macrophage stimulated by microbes
Fibrin and fibronectin (extracellular matrix)

FIGURE 3.3 Multistep leukocyte-endothelial interactions mediating leukocyte recruitment into tissues. *1.* Production of cytokines at site of infection and tissue injury. *2.* Selectin-mediated rolling of leukocytes. *3.* Increase in integrin affinity. *4.* Integrin-mediated firm attachment of leukocytes to endothelium. *5.* Transmigration of leukocytes through endothelium. *6.* Migration of leukocytes to site of infection and tissue injury.

- ***Selectin-mediated rolling of leukocytes on endothelium.*** Macrophages, DCs, and other cells that encounter microbes in extravascular tissues are activated to secrete cytokines, including TNF and IL-1. These cytokines stimulate endothelial cells lining postcapillary venules at the site of an infection to express E-selectin. Endothelial cells also express P-selectin in response to histamine released from microbe-activated mast cells, and thrombin produced during blood coagulation, which occurs commonly in inflammatory reactions. At these sites, blood vessels dilate and blood flow slows. As a result, leukocytes, being larger than red cells, tend to move away from the central axial flow and closer to the vessel lining, a process known as margination. This allows the ligands for E- and P-selectins expressed on the microvilli of the leukocytes to bind to the selectins that have been induced on the endothelial cells. Because the interactions of selectins with their ligands are of low affinity ($K_d \sim 100$ μm) with a fast off-rate, they are easily disrupted by the shear force of the flowing blood. As a result, the selectin–selectin ligand bonds repeatedly form and break, and the leukocytes are pushed along the endothelial surface in a rolling motion. This slowing of leukocytes on the endothelium allows the next set of stimuli in the multistep process to act on the leukocytes.

- ***Chemokine-mediated increase in affinity of integrins.*** Chemokines displayed on endothelial cells of postcapillary venules at the infection site bind to their receptors on the rolling leukocytes. As discussed before, this results in stronger binding of leukocyte integrins to their ligands on the endothelial surface.
- ***Stable integrin-mediated arrest of leukocytes on endothelium.*** In parallel with the activation of integrins, the expression of their ligands on the endothelial cells is upregulated by inflammatory cytokines and microbial products. These ligands include VCAM-1, which binds the integrin VLA-4, and ICAM-1, which binds LFA-1 and MAC-1 integrins. Thus, the leukocytes attach firmly to the endothelium, their cytoskeleton is reorganized, and they spread out on the endothelial surface.
- ***Transmigration of leukocytes through the endothelium.*** Leukocytes usually migrate out of blood vessels between endothelial cells, a process called paracellular transmigration or diapedesis, to reach extravascular tissues. Paracellular transmigration depends on interactions of integrins on the leukocytes and their ligands on the endothelial cells, as well as the contribution of other proteins, notably CD31, which is expressed on leukocytes

and endothelial cells. This process requires a transient and reversible disruption of adherens junction proteins, primarily the VE-cadherin complex, that hold endothelial cells together. The mechanism responsible for disruption of the VE-cadherin complex involves activation of kinases when leukocyte integrins bind ICAM-1 or VCAM-1. The kinases phosphorylate the cytoplasmic tail of VE-cadherin, which leads to reversible disruption of the adherens complex. Less often, leukocytes have been observed to move through endothelial cells rather than between them, by a poorly understood process called transcellular migration.

These basic steps are seen in the migration of all leukocytes through the endothelium. However, neutrophils, monocytes, and different subsets of lymphocytes differ in which tissues they migrate into and when they do so in inflammatory reactions and the steady state. These patterns of leukocyte migration are dependent on the expression of distinct combinations of adhesion molecules and chemokine receptors, as we will discuss in more detail later.

Evidence for the essential role of selectins, integrins, and chemokines in leukocyte migration came first from antibody-blockade studies and gene knockout mice and then from the discovery of rare inherited human diseases called **leukocyte adhesion deficiencies** (see Chapter 21). An autosomal recessive inherited deficiency in the *CD18* gene, which encodes the β subunit of LFA-1, MAC-1, and CD11cCD18, is the cause of an immune deficiency disease called type 1 leukocyte adhesion deficiency (LAD-1), in which there are marked defects in leukocyte migration and immune responses. Patients who lack the Golgi GDP-fucose transporter needed to express the carbohydrate ligands for E-selectin and P-selectin on neutrophils have similar problems, resulting in a syndrome called type 2 leukocyte adhesion deficiency (LAD-2). These disorders are characterized by recurrent bacterial and fungal infections, lack of neutrophil accumulation at sites of infection, and defects in adherence-dependent lymphocyte functions. Rare human mutations in the signaling pathways linking chemokine receptors to integrin activation also result in impaired leukocyte adhesion and recruitment into tissues and therefore ineffective leukocyte defense against infections, a syndrome called type 3 leukocyte adhesion deficiency (LAD-3).

Migration of Neutrophils and Monocytes to Sites of Infection or Tissue Injury

After maturing in the bone marrow, neutrophils and monocytes enter the blood and circulate throughout the body. Although these cells can perform some phagocytic functions within the blood, their main functions, including phagocytosis and destruction of microbes and dead tissue cells, take place in extravascular sites of infection virtually anywhere in the body.

Blood neutrophils and monocytes are recruited to tissue sites of infection and injury by a selectin-, integrin-, and chemokine-dependent multistep process, which follows the basic sequence common to the migration of all leukocytes into tissues, discussed earlier. As we will consider in detail in Chapter 4, neutrophils are usually the most numerous type of leukocyte to accumulate in tissues within 24 to 48 hours after the onset of an infection or injury, and are then replaced by monocytes. This probably reflects the fact that there are many more neutrophils in the blood, and they respond more rapidly to chemokines compared to monocytes or other leukocytes. After entering tissues, neutrophils have short life spans before dying by apoptosis whereas monocytes survive longer and may also proliferate in the tissues. However, in some inflammatory sites, neutrophils are not recruited at all, but monocytes are. These different migratory behaviors likely reflect variations in relative expression of adhesion molecules and chemokine receptors on neutrophils versus monocytes. Neutrophils express CXCR1 and CXCR2, which bind CXCL1 and CXCL8 (IL-8), the major chemokines with ELR motifs that support neutrophil migration into tissues (see Table 3.2). Early neutrophil recruitment is a consequence of early and abundant CXCL8 production by tissue-resident macrophages and other cells in response to infections. In contrast to neutrophils, classical monocytes, which are the main type of monocyte recruited to inflammatory sites, express CCR2. This receptor binds several chemokines, the most important one for monocyte recruitment being CCL2 (MCP-1). Thus, monocyte recruitment occurs when resident tissue cells produce CCL2 in response to infection.

Migration and Recirculation of T Lymphocytes

Lymphocytes are continuously moving through the blood, lymphatic vessels, secondary lymphoid organs, and nonlymphoid tissues, and distinct populations of lymphocytes show different trafficking patterns through these sites (Fig. 3.4) **.** When a mature naive T cell emerges from the thymus and enters the blood, it homes to lymph nodes, spleen, or mucosal lymphoid tissues and migrates into the T cell zones of these secondary lymphoid tissues. If the T cell does not recognize antigen in these sites, it remains naive and leaves the nodes or mucosal tissue through lymphatics and eventually drains back into the blood stream. Once back in the blood, a naive T cell repeats its cycle of homing to secondary lymphoid organs. This trafficking pattern of naive lymphocytes, called lymphocyte recirculation, maximizes the chances that the small number of naive lymphocytes that are specific for a particular foreign antigen will encounter that antigen if it shows up anywhere in the body. Lymphocytes that have recognized and become activated by antigen within secondary lymphoid organs proliferate and differentiate to produce thousands of effector and memory cells. The effector and memory lymphocytes may move back into the blood stream and then migrate into sites of infection or inflammation in nonlymphoid tissues.

Some effector and memory lymphocyte subsets preferentially home to a particular tissue, such as skin or gut (see Chapter 14). The existence of different homing patterns ensures that different subsets of lymphocytes that are specialized to defend against different types of microbes, such as parasites, extracellular fungi and bacteria, or intracellular bacteria, are delivered to the tissues where those microbes are and not, wastefully, to places where they would serve no purpose.

In the following section, we describe the mechanisms and pathways of lymphocyte recirculation and homing. Our discussion emphasizes T cells because more is known about their movement through tissues than is known about B cell recirculation, but many of the same mechanisms appear to mediate the recirculation of both cell types.

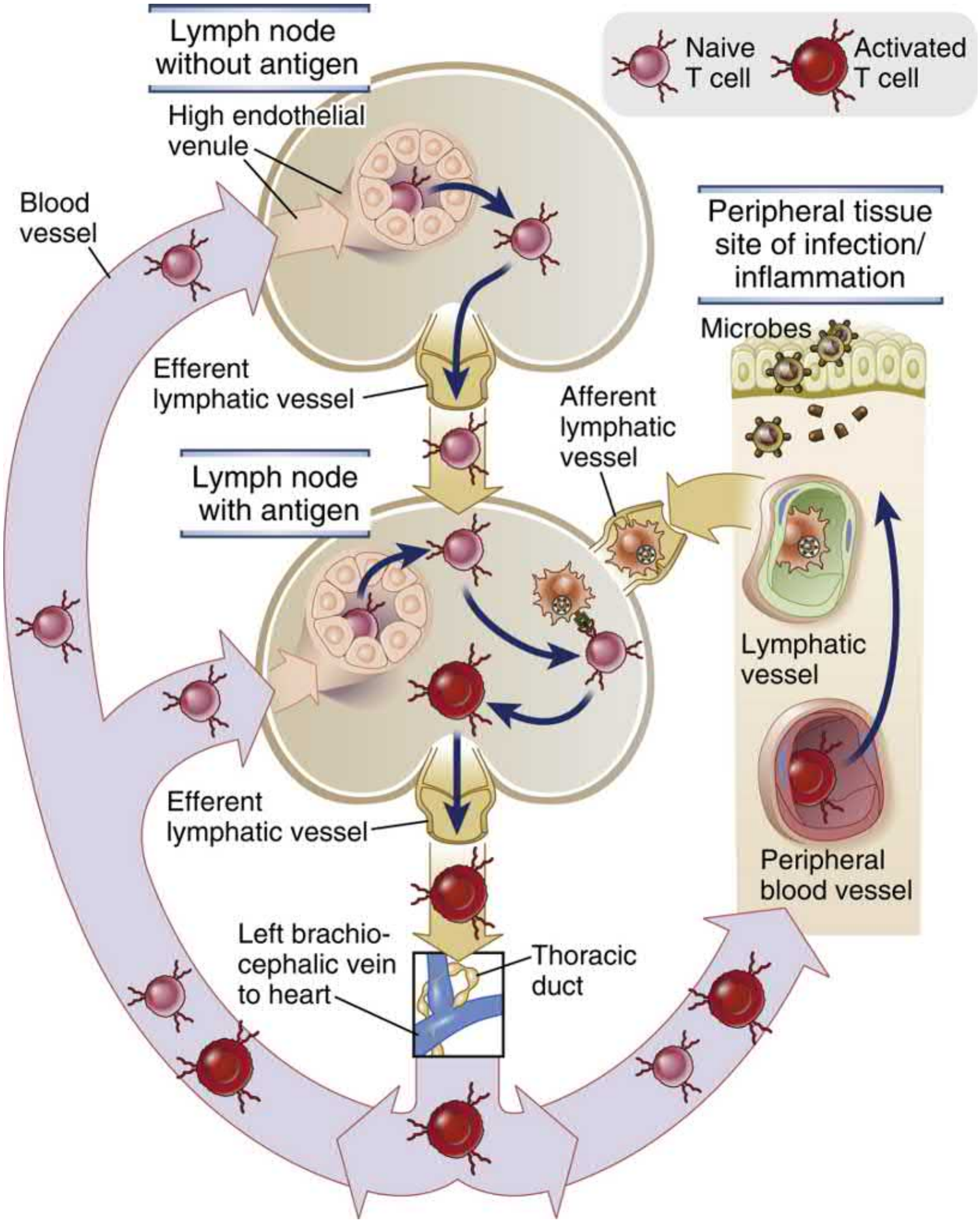

Lymph node without antigen
Naive T cell
Activated T cell
High endothelial venule
Blood vessel
Peripheral tissue site of infection/ inflammation
Microbes
Efferent lymphatic vessel
Afferent lymphatic vessel
Lymph node with antigen
Lymphatic vessel
Efferent lymphatic vessel
Peripheral blood vessel
Left brachio-cephalic vein to heart
Thoracic duct

FIGURE 3.4 Pathways of T lymphocyte recirculation.Naive T cells preferentially leave the blood and enter lymph nodes across the high endothelial venules. Dendritic cells bearing antigen enter the lymph nodes through lymphatic vessels. Naive T cells that do not recognize antigen after several hours return to the circulation. If the T cells recognize antigen, they are activated and

generate effector and memory T cells, which also leave the lymph node and return to the circulation. Effector and memory T cells preferentially leave the blood and enter peripheral tissues through venules at sites of inflammation. Recirculation through secondary lymphoid organs other than lymph nodes is not shown.

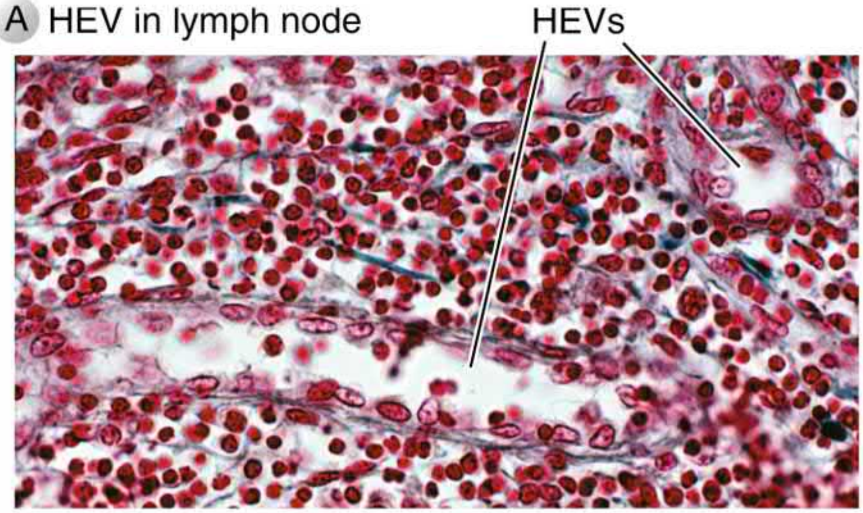
Recirculation of Naive T Lymphocytes Between Blood and Secondary Lymphoid Organs

T lymphocyte recirculation depends on mechanisms that control entry of naive T cells from the blood into lymph nodes, as well as molecular signals that control the exit of naive T cells from the nodes. We will discuss these two mechanisms separately.

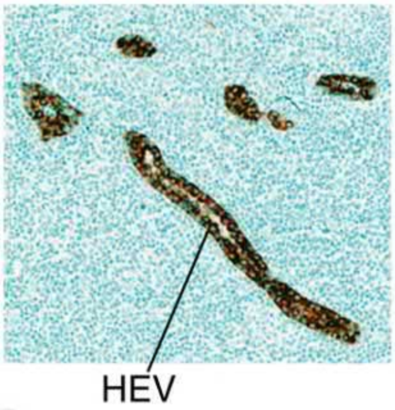
Migration of Naive T Cells Into Lymph Nodes

The homing mechanisms that bring naive T cells into lymph nodes are very efficient, resulting in a net flux of lymphocytes through lymph nodes that is estimated to be up to 25×10^9 cells each day. On average, every naive T lymphocyte in the body goes through at least one node once a day. Peripheral tissue inflammation, which usually accompanies infections, causes a significant increase of blood flow into lymph nodes draining the affected tissue and consequently an increase in T cell influx into those lymph nodes. At the same time, egress of the T cells into efferent lymphatics is transiently reduced by mechanisms we will discuss later, so that T cells stay in lymph nodes that drain sites of inflammation longer than in other lymph nodes. Protein antigens are concentrated in the lymph nodes and other secondary lymphoid organs, where they are presented to T cells by DCs, the type of APCs best able to initiate responses of naive T cells (see Chapter 6). Thus, movement and transient retention of naive T cells in the secondary lymphoid organs, together with capture and concentration of antigen, maximize the chances of T cell activation and initiation of an adaptive immune response.

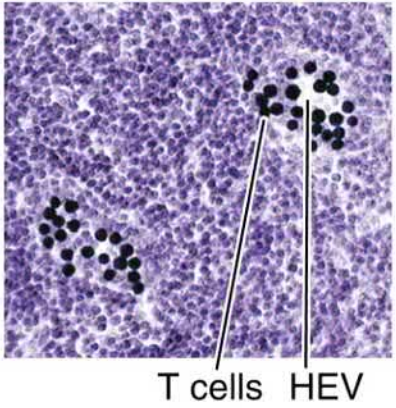
A HEV in lymph node



B L-selectin ligand on endothelial cells



C T cells binding to HEV: frozen section assay



D T cells binding to HEV: electron micrograph

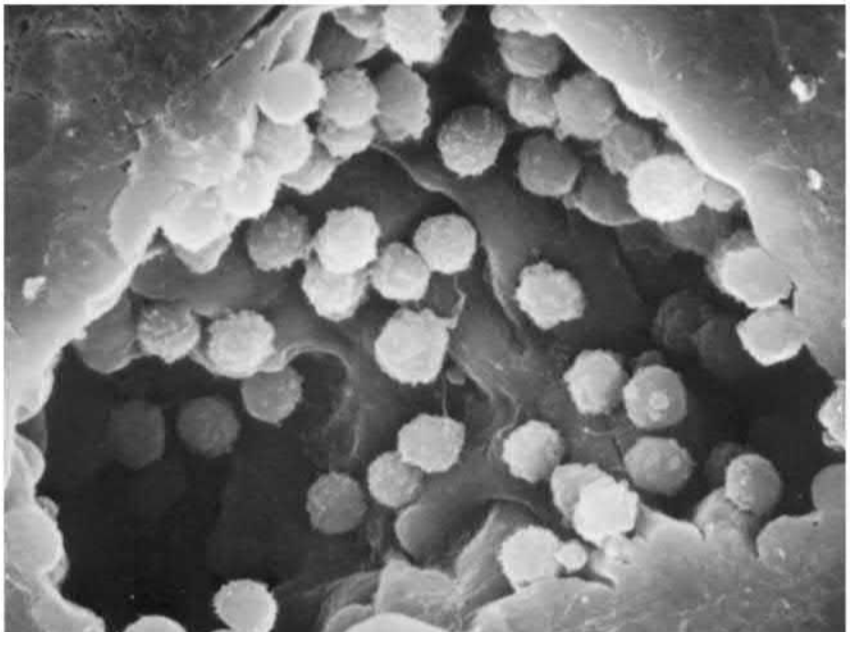


FIGURE 3.5 High endothelial venules. **A,** Light micrograph of ahigh endothelial venules *(HEVs)* in a lymph node, illustrating the tall endothelial cells. **B,** Expression of L-selectin ligand on HEVs, stained with a specific antibody by the immunoperoxidase technique. (The location of the antibody is revealed by a brown reaction product of peroxidase, which is coupled to the antibody; see Appendix III for details.) The HEVs are abundant in the T cell zone of the lymph node. **C,** A binding assay in which lymphocytes are incubated with frozen sections of a lymph node. The lymphocytes *(stained dark blue)* bind selectively to HEVs. **D,** Scanning electron micrograph of an HEV with lymphocytes attached to the luminal surface of the endothelial cells.

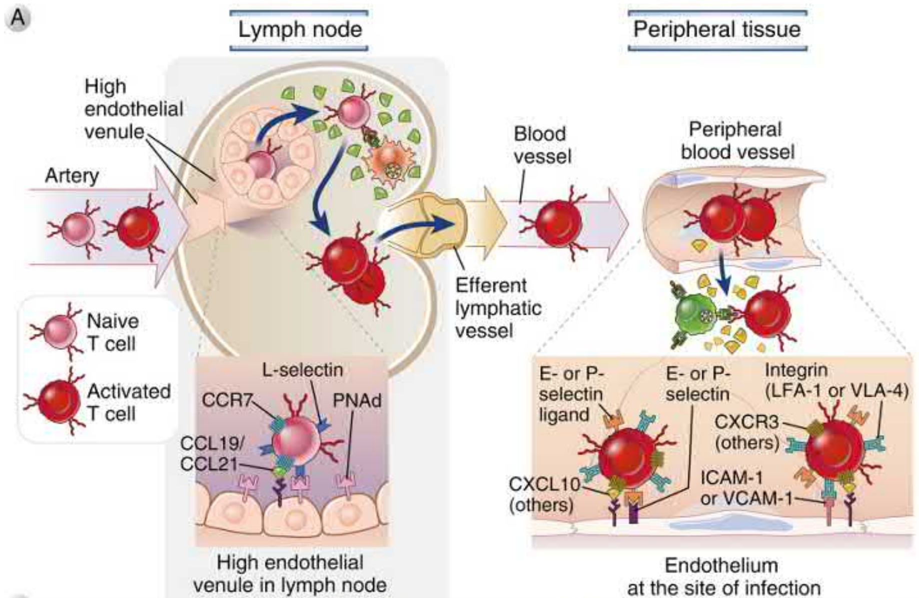
A, Courtesy of Dr. Steve Rosen, Department of Anatomy, University of California, San Francisco. *B,* Courtesy of Drs. Steve Rosen and Akio Kikuta, Department of Anatomy, University of California, San Francisco. *C,* Courtesy of Dr. Steve Rosen, Department of Anatomy, University of California, San Francisco. *D,* Courtesy of J. Emerson and T. Yednock, University of California, San Francisco, School of Medicine. From Rosen SD, Stoolman LM. Potential role of cell surface lectin in lymphocyte recirculation. In: Olden K, Parent J, eds. *Vertebrate Lectins.* New York, NY: Van Nostrand Reinhold; 1987.

Homing of naive T cells into lymph nodes and mucosa-associated lymphoid tissues (MALTs) occurs through specialized postcapillary HEVs located in the T cell zones. Naive T lymphocytes are delivered to secondary lymphoid tissues through arterial blood flow, and they leave the circulation and migrate into the stroma of lymph nodes through these HEVs. These vessels are lined with plump endothelial cells and not the flat endothelial cells that are typical of other venules (Fig. 3.5). HEVs are also present in mucosal lymphoid tissues, such as Peyer's patches in the gut, but not in the spleen. The endothelial cells of HEVs are specialized to display certain adhesion molecules and chemokines on their surfaces, discussed later, which support the selective homing of only certain populations of lymphocytes. Certain cytokines, such as lymphotoxin, are required for HEV development. In fact, HEVs may develop in extralymphoid sites of chronic inflammation, where such cytokines are produced for prolonged periods.

Naive T cell migration out of the blood through the HEVs into the lymph node parenchyma involves the adhesion molecules L-selectin and LFA-1 and the chemokine receptor CCR7. This process includes the sequential events described earlier for migration of all leukocytes (see Fig. 3.3), but migration across HEVs into lymphoid tissues involves particular adhesion molecules and chemokines (Fig. 3.6).

- The rolling of naive T cells on HEVs in secondary lymphoid organs is mediated by L-selectin on the lymphocytes binding to PNAd on the HEV. PNAd is a sulfated 6 sialyl Lewis X carbohydrate attached to a glycoprotein backbone. The PNAd carbohydrate group that binds L-selectin may be attached to different sialomucins on the HEVs in different tissues. For example, on lymph node HEVs, the L-selectin ligand is displayed by two sialomucins, called glycan-bearing cell adhesion molecule 1 (GlyCAM-1) and CD34. In Peyer's patches in the intestinal wall, the L-selectin ligand is exhibited on a glycoprotein called MAdCAM-1, which is also the ligand for the $\alpha_4\beta_7$ integrin.
- As with leukocyte migration in other sites, the subsequent firm adhesion of the naive T cells to the HEVs is mediated by integrins, mainly LFA-1.
- The principal chemokines that activate the naive T cell integrins to a high-affinity state are CCL19 and CCL21, which are uniquely involved in leukocyte homing to T cell zones of lymphoid tissues (see Chapter 2). The main source

of CCL19 and CCL21 is fibroblast reticular cells (FRCs) within the T cell zone, and CCL21 is also constitutively produced by HEVs. These chemokines are displayed on the surface of the HEV and recognized by rolling lymphocytes. Both these chemokines bind to the chemokine receptor CCR7, which is expressed at high levels on naive T cells. This interaction of the chemokines with CCR7 ensures that naive T cells increase integrin avidity and are able to adhere firmly to HEVs. Recall that CCR7 also governs DC migration via lymphatics into lymph nodes. The important role of L-selectin and chemokines in naive T cell homing to secondary lymphoid organs is supported by many different experimental observations. Lymphocytes from L-selectin knockout mice do not bind to peripheral lymph node HEVs, and the mice have a marked reduction in the number of lymphocytes in lymph nodes. There are very few naive T cells in the lymph nodes of mice with genetic deficiencies in CCL19 and CCL21 or CCR7.


A
Lymph node
Peripheral tissue
High endothelial venule
Artery
Blood vessel
Peripheral blood vessel
Efferent lymphatic vessel
Naive T cell
Activated T cell
L-selectin
CCR7
PNAd
CCL19/ CCL21
High endothelial venule in lymph node
E- or P-selectin ligand
E- or P-selectin
Integrin (LFA-1 or VLA-4)
CXCR3 (others)
CXCL10 (others)
ICAM-1 or VCAM-1
Endothelium at the site of infection

B

T cell homing receptor	Ligand on endothelial cell	Function of receptor: ligand pair
Naive T cells		
L-selectin	PNAd	Initial weak adhesion of naive T cells to high endothelial venule in lymph node
CCR7	CCL19 or CCL21	Activation of integrins and chemokinesis
LFA-1 (β2-integrin)	ICAM-1	Stable arrest on high endothelial venule in lymph node
Activated (effector and memory) T cells		
E- and P-selectin ligand	E- or P-selectin	Initial weak adhesion of effector and memory T cells to cytokine activated endothelium at peripheral site of infection
CXCR3	CXCL10 (others)	Activation of integrins and chemokinesis
CCR5	CCL4 (others)	Activation of integrins and chemokinesis
LFA-1 (β2-integrin) or VLA-4 (β1 integrin)	ICAM-1 or VCAM-1	Stable arrest on cytokine activated endothelium at peripheral site of infection

FIGURE 3.6 Molecules involved in migration of naive and effector T lymphocytes. **A,** Naive T lymphocytes home to lymph nodes as a result of L-selectin binding to peripheral node addressin (PNAd) on HEVs, which are present only in secondary lymphoid organs, and as a result of binding chemokines (CCL19 and CCL21) displayed on the surface of the HEV. Activated T lymphocytes, including effector cells, home to sites of

infection in peripheral tissues, and this migration is mediated by E-selectin and P-selectin, integrins, and chemokines that are produced at sites of infection. Additional chemokines and chemokine receptors, besides the ones shown, are involved in effector/memory T cell migration. **B,** The adhesion molecules, chemokines, and chemokine receptors involved in naive and effector/memory T cell migration are described. *ICAM-1,* Intercellular adhesion molecule 1; *LFA-1,* leukocyte function–associated antigen 1; *VCAM-1,* vascular cell adhesion molecule 1; *VLA-4,* very late antigen 4.

Movement of T Cells Within Secondary Lymphoid Organs

After entering lymph nodes or mucosal lymphoid tissues, T lymphocytes and DCs actively move in ways that maximize the chances of the two cell types interacting with one another. Initiation of T cell–mediated immune responses requires that rare antigen-specific naive T cells recognize specific antigens displayed on the surface of DCs, which are also numerically rare. Although their shared expression of CCR7 promotes the localization of both these cell types to the same area of the lymph node or mucosal lymphoid tissue, there are thousands of DCs and naive T cells in these locations. However, naive T cells are remarkably motile within the lymph node, moving like amoebas up to 12 µm/minute. T cell movement is stimulated by CCL21 binding to CCR7 on the T cells. The FRCs that secrete CCL19 and CCL21 form three-dimensional networks that traverse the T cell zones of the lymph node, and the T cells move along these FRC tracks. DCs are also distributed along the FRC network, covering most of the surface, and, although they are not highly motile, they constantly extend their dendrites in different directions. As a result, each DC can contact many T cells over time, as many as ~85/minute. A single naive T cell may move along the FRC network in one lymph node for up to 24 hours, and, therefore, if there are some DCs displaying a particular antigen in a lymph node, a naive T cell specific for that antigen that enters the node will likely find one of the those DCs. Immediately after recognizing antigen on a DC, the T cell stops moving and the interaction with the DC is stabilized, which allows for full activation of the T cell (see Chapter 9).

Exit of Naive and Effector T Cells From Lymph Nodes

Naive T cells that have homed into lymph nodes but fail to recognize antigen and are not activated will eventually return to the blood stream. This return to the blood completes one recirculation loop and provides the naive T cells another chance to enter secondary lymphoid organs and search for the antigens they can recognize. The major route of reentry into the blood is through the efferent lymphatics, perhaps via other lymph nodes in the same chain, then through the lymphatic vasculature to the thoracic duct, which drains into the superior vena cava, or to the right lymphatic duct, which drains into the right subclavian vein.

The egress of naive T cells from lymph nodes is dependent on a lipid chemoattractant called sphingosine 1-phosphate (S1P), which binds to a GPCR family receptor on T cells called sphingosine 1-phosphate receptor 1 (S1PR1) (Fig. 3.7) **.** S1P is present at higher concentrations in the blood and lymph than in tissues. This concentration gradient is maintained because an S1P-degrading enzyme, S1P lyase, is present in most tissues, so the lipid is catabolized in tissues more than in the lymph and blood. S1PR1 stimulates migration of cells toward a gradient of S1P. Circulating naive T cells have very little surface S1PR1 because the high blood concentration of S1P causes internalization of the receptor. After a naive T cell enters a lymph node, where S1P concentrations are low, S1PR1 reappears on the cell surface over a period of several hours. This time lag allows a naive T cell to interact with APCs. If the naive

T cell does not recognize any antigens before the S1PR1 receptor is re-expressed, the T cell leaves the lymph node and is directed down the S1P concentration gradient into the efferent lymphatic, and eventually returns to the blood.

The egress from lymph nodes of effector T cells, generated from antigen-activated naive T cells, is also dependent on S1P. When a naive T cell is activated by antigen in the lymph node, the surface expression of S1PR1 is suppressed for several days, and, therefore, the ability of the cells to leave the lymphoid organ in response to an S1P gradient is delayed. This suppression of S1PR1 is controlled in part by cytokines called type I interferons that are produced during innate immune responses to infections, as we will discuss in Chapter 4. Antigenic stimulation and interferons together increase the expression of a protein called CD69, which binds to intracellular S1PR1 and reduces its cell surface expression. Thus, the activated T cell becomes transiently insensitive to the S1P gradient. This allows the antigen-activated T cells to remain in the lymphoid organ and undergo clonal expansion and differentiation into effector T cells, a process that may take several days. When differentiation into effector cells is complete, the cells stop expressing CD69, and, therefore, S1PR1 is again expressed on the cell surface. At the same time, the newly generated effector cells reduce expression of L-selectin and CCR7, which previously attracted the naive T cells to the lymph nodes. Therefore, the effector T cells become responsive to the concentration gradient of S1P and exit the lymph node via the medullary sinus draining into the efferent lymphatic, and eventually re-enter the blood. S1P and the S1PR1 are also required for mature T cell egress from the thymus and migration of B cell–derived plasmablasts from secondary lymphoid organs.

Our understanding of the role of S1P and S1PR1 in T cell trafficking is based in part on studies of a drug called fingolimod (FTY720), which binds to S1PR1 and causes its down-modulation from the cell surface. Fingolimod blocks T cell egress from lymphoid organs and thereby acts as an immunosuppressive drug. It is approved for the treatment of multiple sclerosis, an autoimmune disease of the central nervous system, and the use of fingolimod and other drugs with a similar mechanism of action to treat other autoimmune diseases and graft rejection is under investigation. Additional experimental evidence for the central role of S1P in naive T cell trafficking comes from studies of mice with genetic ablation of S1PR1. In these mice, there is failure of T cells to leave the thymus and populate secondary lymphoid organs. If naive T cells from S1PR1 knockout mice are injected into the circulation of other mice, the cells enter the lymph nodes but are unable to exit.

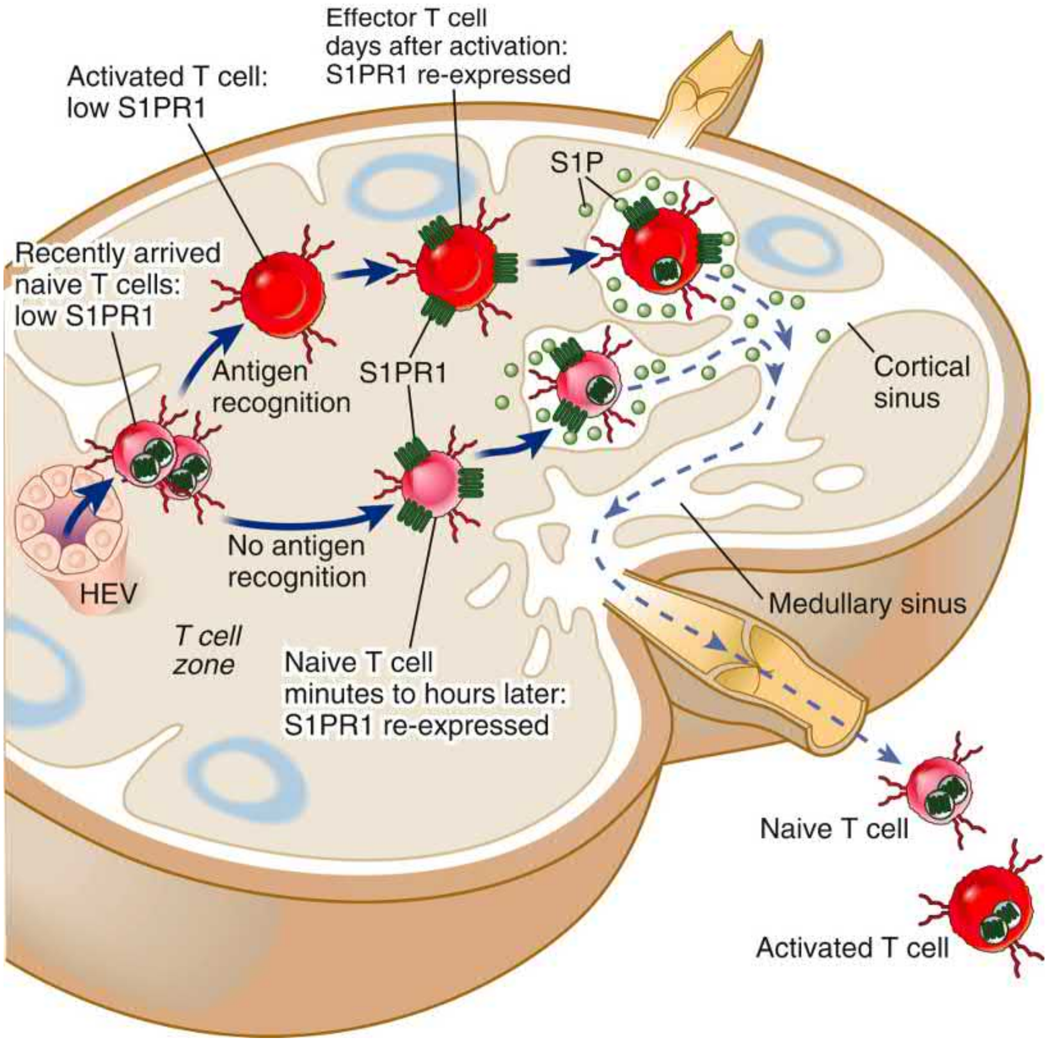

Effector T cell
days after activation:
S1PR1 re-expressed
Activated T cell:
low S1PR1
S1P
Recently arrived
naive T cells:
low S1PR1
Antigen
recognition
S1PR1
Cortical
sinus
HEV
No antigen
recognition
Medullary sinus
T cell
zone
Naive T cell
minutes to hours later:
S1PR1 re-expressed
Naive T cell
Activated T cell

FIGURE 3.7 Mechanism of egress of lymphocytes from lymphoid organs.S1P is present at relatively high concentration in the blood and lymph and at lower concretions within lymphoid tissues. Circulating naive T cells have low levels of S1PR1 because the receptor is internalized after binding S1P in the blood. Therefore, naive T cells that have recently entered a lymph node initially cannot sense the S1P concentration gradient between the T cell zone of the node and the lymph in the medullary sinus and efferent lymphatics, and these T cells cannot exit the node. After activation of a naive T cell by antigen, surface expression of S1PR1 is blocked for several days, and the

activated cells also will not leave the node. S1PR1 is re-expressed after several minutes to hours for naive T cells, or days for activated and differentiated effector T cells, and these cells can then sense the S1P gradient and exit the node. The average dwell time is about 12 hours for naive T cells in lymph nodes and about 3 days for antigen-stimulated T cells, but this varies greatly in different conditions.

Recirculation of T Cells Through Other Lymphoid Tissues

Naive T cell homing into gut-associated lymphoid tissues, including Peyer's patches and mesenteric lymph nodes, is fundamentally similar to homing to other lymph nodes and relies on interactions of the T cells with HEVs. As in other tissues, these interactions are mediated by selectins, integrins, and chemokines. One particular feature of naive T cell homing to mesenteric lymph nodes and Peyer's patches is the contribution of the Ig superfamily molecule MAdCAM-1, which is expressed on HEVs in these sites but not typically elsewhere in the body. The two ligands on naive T cells that bind to MAdCAM-1, L-selectin and the integrin $\alpha_4\beta_7$, both contribute to the homing of naive T cells into gut-associated lymphoid tissues.

Naive T cell migration into the spleen through the splenic white pulp differs from migration into lymph nodes. There are no HEVs in the spleen, and there appears to be unregulated T cell entry through the open circulation. T cells are then directed into a T cell zone of the white pulp by CCR7-binding chemokines. The rate of lymphocyte passage through the spleen is very high, approximately half the total circulating lymphocyte population every 24 hours. T cell egress from white pulp to red pulp and the circulation is dependent on S1P and S1PR1.

Migration of Effector T Lymphocytes to Sites of Infection

Circulating effector T cells preferentially home to peripheral tissue sites of infection rather than lymphoid organs, because of changes in adhesion molecule and chemokine receptor expression. Many of the protective antimicrobial functions of effector T cells must be performed locally at sites of infections (see Chapters 10 and 11), and, therefore, these cells must be able to leave lymphoid organs and migrate into infected tissues. After differentiation of naive T lymphocytes into effector cells, when S1PR1 expression is restored and expression of CCR7 and L-selectin is lost, the effector T cells also start expressing receptors for inflammatory chemokines produced at infection sites and the ligands for E- and P-selectins. These changes impair homing of the effector T cells into other secondary lymphoid organs and promote their migration into sites of infection.

The process of effector lymphocyte homing into infected tissues occurs in postcapillary venules and is mediated by the same multistep selectin-, integrin-, and chemokine-dependent process described for other leukocytes. As with neutrophils and monocytes, effector T cells in the circulation, but not naive T cells, express selectin ligands, integrins, and chemokine receptors that bind to the types of selectins, integrin ligands, and chemokines, respectively, that are expressed in activated endothelium (see Fig. 3.6).

The migration of effector T cells into infected tissues is antigen-independent, but the effector cells that encounter antigen in the tissue are preferentially retained there. Thus, effector cells of diverse specificities can enter tissue sites of infection, which maximizes the chance of cells finding the antigen for which they are specific. On effector T cells that recognize antigen in infected tissues, the integrins are kept in their high-affinity state because of antigen-induced activation and the continued presence of chemokines. These integrins bind tightly to extracellular matrix proteins, and this favors retention of the effector T cells that encounter antigens at these sites. Retention allows effector T cells that recognize antigens to perform the functions that eliminate microbes and other sources of the antigens. Most effector cells that enter a site of infection eventually die there after performing their functions.

Some effector cells have a propensity to migrate to particular types of tissues. This selective migration capacity is acquired during the differentiation of the effector T cells from naive precursors in secondary lymphoid organs. By enabling distinct groups of effector T cells to migrate to different sites, the adaptive immune system directs cells with specialized effector functions to the locations where they are best suited to deal with particular types of infections. The clearest examples of populations of effector T cells that specifically home to different tissues are skin-homing and gut-homing T cells, whose migration patterns reflect the expression of different adhesion molecules and chemokine receptors on each subset, discussed in detail in Chapter 14.

Different subsets of effector T cells exist, each with distinct functions, and these subsets have different although often overlapping patterns of migration. Effector T cells include CD8$^+$ cytotoxic T lymphocytes and CD4$^+$ helper T cells. Helper T cells include Th1, Th2, and Th17 subsets, each of which expresses different types of cytokines and protects against different types of microbes. The characteristics and functions of these subsets will be discussed in detail in Chapter 10. For now, it is sufficient to know that the migration of these subsets shows some differences. This is because the array of chemokine receptors and adhesion molecules expressed by each subset differs in ways that result in preferential recruitment of each subset into sites of inflammation elicited by different types of infections.

Memory T Cell Migration

Memory T cells are heterogeneous in their patterns of expression of adhesion molecules and chemokine receptors and in their propensity

to migrate to different tissues. Because the ways of identifying memory T cells are still imperfect (see Chapters 2 and 9), the distinction between effector and memory T cells in experimental studies and in humans is often not precise. Two subsets of memory T cells, called central memory and effector memory T cells, were initially identified on the basis of differences in CCR7 and L-selectin expression. **Central memory T cells** (T_{CM}) were defined as human CD45RO$^+$ blood T cells that express high levels of CCR7 and L-selectin; **effector memory T cells** (T_{EM}) were defined as CD45RO$^+$ blood T cells that express low levels of CCR7 and L-selectin but express other chemokine receptors that bind inflammatory chemokines. These phenotypes suggest that central memory T cells home to secondary lymphoid organs, whereas effector memory T cells home to peripheral tissues. Although central and effector memory T cell populations also can be detected in mice, experimental studies have indicated that CCR7 expression is not a definitive marker to distinguish these memory T cell subpopulations. Nonetheless, it is clear that some memory T cells tend to home to secondary lymphoid organs, whereas others migrate into peripheral tissues, especially skin and mucosal tissues. Many of these cells recirculate between blood and secondary lymphoid organs, like naive T cells, or between blood and skin or mucosal tissues.

Some memory T cells migrate into tissues after an infection and remain there indefinitely. These non-recirculating T cells, called **tissue-resident memory T cells** (or T_{RM}), stay in peripheral tissues and secondary lymphoid organs for months to years after infections without recirculation into blood. Both CD4$^+$ and CD8$^+$ tissue-resident memory T cells have been found in many types of normal and disease tissues in mice and humans, including skin, lung, liver, intestines, brain, lymph nodes, and spleen. A common feature of these cells is the expression of molecules that promote retention in tissues. Most tissue-resident memory T cells express CD69, which as discussed earlier blocks surface expression of S1PR1, thereby conferring resistance of these cells to S1P-mediated egress from tissues. Many tissue-resident memory T cells also express the integrin CD103, which binds to E-cadherin, a molecule expressed by epithelial cells. Based on experimental models in mice and data from human tissue analyses, the presence of tissue-resident memory T cells is associated with resistance to reinfection by microbes.

Migration of B Lymphocytes

B lymphocyte migration is critical for effective adaptive immunity, although the patterns of migration differ from those of T cells. The initiation of B cell responses to infection begins in secondary lymphoid organs, and often depends on help from T cells. Therefore, this first stage of a B cell response requires similar migratory activities of naive B cells as we described for naive T cells. However, secreted antibodies perform the effector functions of B cells at sites distant from the B cells, and these antibodies, but not the B cells themselves, need to be delivered by the blood to infected tissues. Some of the antibody-secreting B cells generated from naive B cells in lymph nodes or spleen do migrate to bone marrow or mucosal sites, where they remain and secrete antibodies for long periods.

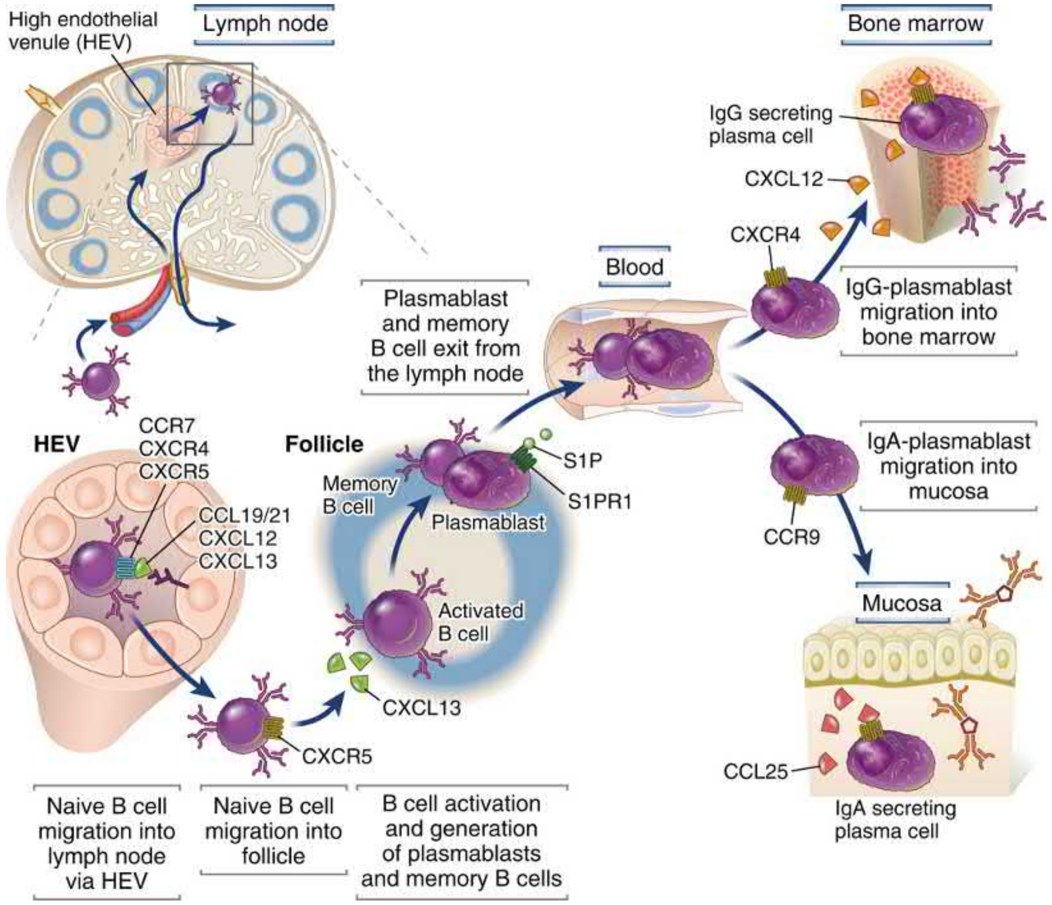

High endothelial venule (HEV)
Lymph node
Bone marrow
IgG secreting plasma cell
CXCL12
CXCR4
Blood
IgG-plasmablast migration into bone marrow
Plasmablast and memory B cell exit from the lymph node
HEV
CCR7
CXCR4
CXCR5
Follicle
S1P
S1PR1
Memory B cell
Plasmablast
CCL19/21
CXCL12
CXCL13
IgA-plasmablast migration into mucosa
CCR9
Activated B cell
Mucosa
CXCL13
CXCR5
CCL25
IgA secreting plasma cell
Naive B cell migration into lymph node via HEV
Naive B cell migration into follicle
B cell activation and generation of plasmablasts and memory B cells

FIGURE 3.8 Migration of B cells.Naive B cells enter lymph nodes and mucosal-associated lymphoid tissues through high endothelial venules (HEVs), migrate into follicles, become activated, and differentiate into antibody-producing cells, some of which are plasmablasts that enter the circulation and migrate into bone marrow or mucosal tissues, where they fully differentiate into plasma cells. Immunoglobulin G *(IgG)*-secreting plasma cells may be generated in any lymphoid tissues. *IgA*-secreting plasma cells are produced mainly in mesenteric lymph nodes or mucosa-associated lymphoid tissues and home back to mucosal tissues. Other B cells that enter follicles differentiate into memory B cells, some of which enter the circulation. The chemokine receptors

and chemokines involved in these steps are shown. Adhesion molecules are also involved in migration out of the HEV and blood vessels in tissues, as described in the text.

Recirculation of Naive Follicular B Cells

Naive follicular B cells use the same basic mechanisms as do naive T cells to home to and exit out of secondary lymphoid organs. There are different subsets of B cells, but the major subset responsible for most antibody production are called follicular B cells, and we will describe their migratory activities. Immature follicular B cells leave the bone marrow through the blood, enter the spleen through the marginal zone, and migrate to the periphery of the white pulp. As they mature further, the B cells express the chemokine receptor CXCR5, which promotes their movement into the white pulp in response to a chemokine called CXCL13 that is produced by follicular dendritic cells (FDCs) in lymphoid follicles. After the maturation is completed within the white pulp, naive follicular B cells reenter the circulation by an S1P-driven process and then recirculate to lymph nodes, mucosal lymphoid tissues, or back to the spleen. Homing of naive B cells from the blood into lymph nodes and Peyer's patches in the small bowel involves rolling interactions on HEVs, chemokine activation of integrins, and stable arrest, as described earlier for naive T cells (Fig. 3.8). This process depends on the chemokine receptors CCR7, CXCR4, and CXCR5 on naive B cells and their respective ligands CCL19/CCL21, CXCL12, and CXCL13. CCL19/CCL21 and CXCL12 are displayed by HEVs in lymph nodes and mediate the entry of naive and memory B cells into lymph nodes. CXCL13 then draws B cells into the B cell zone, the follicles. There are no HEVs in the white pulp of the spleen, and the mechanisms of homing of naive B cells into splenic white pulp are not well understood. Homing of naive B cells into Peyer's patches in the small bowel wall involves CXCR5 and the integrin $\alpha_4\beta_7$, which binds to MAdCAM-1. During the course of B cell responses to protein antigens, B cells and helper T cells must directly interact, and this is made possible by highly regulated movements of both cell types within the secondary lymphoid organs. These local migratory events, and the chemokines that orchestrate them, will be discussed in detail in Chapter 12. If a naive B cell is not activated by antigen in a lymph node or the spleen after several hours, it will respond to the S1P gradient, exit into the circulation, and recirculate back into other secondary lymphoid organs. B cells in the lymph node will exit through efferent lymphatics and by the thoracic duct into the blood, and B cells in the spleen migrate to the marginal zone and then are carried by fluid through the red pulp into the circulation.

Migration of Antibody Secreting Plasmablasts and Memory B Cells

Plasmablasts derived from follicular B cells exit out of secondary lymphoid organs and home to bone marrow or mucosal tissues, where they differentiate into long-lived plasma cells and secrete antibodies (see Fig. 3.8). If a naive B cell is activated by antigen in a secondary lymph node, it will differentiate into antibody-secreting cells called plasmablasts. Some of these plasmablasts will mature in the secondary lymphoid organ itself into plasma cells with short life spans that will secrete antibodies locally. However, as a response to a protein antigen progresses, and T cells help B cells (described in Chapter 12), plasmablasts are generated in the germinal centers of follicles. These plasmablasts exit the secondary lymphoid tissues by an S1P-dependent mechanism and enter the blood. Many of these cells home to the bone marrow, and some to mucosal tissues, where they mature into long-lived plasma cells that stay in these tissues and secrete antibodies for long periods.

Most memory B cells are generated in germinal centers of secondary lymphoid organs along with plasmablasts. Some memory B cells migrate to bone marrow and mucosal sites with the plasmablasts, whereas other memory B cells appear to recirculate through secondary lymphoid organs. Reactivation of memory B cells upon re-exposure to microbial protein antigens induces new germinal center reactions in the lymphoid organs. Overall, the mechanisms that govern memory B cell migration and recirculation are not well understood.

Subsets of activated B cells committed to producing particular types of antibodies migrate from secondary lymphoid organs into specific tissues, where they differentiate into long-lived plasma cells (see Fig. 3.8) **.** As we will describe in later chapters, during their responses to antigen, B cells may differentiate into cells that produce different types of antibodies, called isotypes (or classes), each of which performs a distinct set of effector functions. The genetic changes that determine which isotype will be expressed by B cells occur before differentiation into plasmablasts and finally into plasma cells. B cells that are committed to secreting antibodies of the IgG isotype will differentiate into plasmablasts that mostly migrate to the bone marrow, and accordingly, most plasma cells residing in the bone marrow produce IgG antibodies, which are then distributed throughout the body via the blood stream. B cells that are activated within MALTs usually become committed to expression of the IgA isotype of antibody, and IgA-producing plasmablasts home specifically to mucosal tissues. The local differentiation within the mucosal lymphoid tissues of B cells into IgA-secreting cells, combined with the homing of these cells into the mucosa, optimizes IgA defense against microbial invasion through the mucosal barriers. As we will discuss in more detail in Chapter 14, IgA is efficiently secreted into the lumen of tissues lined by mucosal epithelia, such as the gut and respiratory tract.

The mechanisms by which different B cell populations migrate to different tissues are similar to the mechanisms we described for tissue-specific migration of effector T cells and depend on expression of distinct combinations of adhesion molecules and chemokine receptors on each B cell subset. For example, bone marrow–homing IgG-secreting plasma cells express VLA-4 and CXCR4, which bind respectively to VCAM-1 and CXCL12 expressed on bone marrow sinusoidal endothelial cells. In contrast, mucosa-homing IgA-secreting plasma cells express $\alpha_4\beta_7$ and CCR9, which bind respectively to MAdCAM-1 and CCL25 on mucosal endothelial cells. IgG-secreting B cells are also recruited to chronic inflammatory sites in various tissues, and this homing pattern can be attributed to VLA-4 and CXCR3 on these B cells binding to VCAM-1, CXCL9, and CXCL10, which are often found on the endothelial surface at sites of chronic inflammation.

Summary

- Leukocyte migration from blood into tissues occurs through postcapillary venules and depends on chemokines and adhesion molecules expressed on the leukocytes and vascular endothelial cells.
- Selectins are carbohydrate-binding adhesion molecules that mediate low-affinity interaction of leukocytes with endothelial cells, the first step in leukocyte migration from blood into tissues. E-selectin and P-selectin are expressed on activated endothelial cells and bind to selectin ligands on leukocytes, and L-selectin is expressed on leukocytes and binds ligands on endothelial cells.
- Integrins are a large family of adhesion molecules, some of which mediate tight adhesion of leukocytes with activated endothelium, a critical step in leukocyte migration from blood into tissues. The important leukocyte integrins include LFA-1 and VLA-4, which bind to ICAM-1 and VCAM-1, respectively, on endothelial cells. Chemokines and other signals at sites of infection increase the affinity of integrins on leukocytes, and various cytokines (tumor necrosis factor, interleukin-1) increase the expression of integrin ligands on endothelium.
- Chemokines are a family of proteins that regulate when and how leukocytes migrate into and within tissues, and they organize the functional locations of lymphocytes and dendritic cells (DCs) in lymphoid organs. Chemokines bind

to chemokine receptors on leukocytes, which signal to increase leukocyte integrin affinity and stimulate leukocyte chemokinesis along a concentration gradient of chemokines. Different types of leukocytes and leukocytes at different stages of differentiation express distinct sets of chemokine receptors, and the types of chemokines present in tissues or on endothelial cells varies with different inflammatory states and tissue types.

- Migration of leukocytes from blood into tissues involves a series of sequential interactions with endothelial cells, starting with low-affinity leukocyte binding to and rolling along the endothelial surface (mediated by selectins and selectin ligands). Next, chemokines displayed on endothelial cells bind to chemokine receptors on the rolling leukocytes, which generates signals that increase the affinity of leukocyte integrins. Then the leukocytes become firmly bound to the endothelium through interactions of the integrins binding to Ig superfamily ligands on the endothelium. Finally, the leukocytes move through cell junctions between endothelial cells into the tissues.
- Lymphocyte recirculation is the process by which naive lymphocytes continuously migrate from the blood into the secondary lymphoid organs, back into the blood through lymphatics, and into other secondary lymphoid organs. This process maximizes the chance of naive T or B cell encounter with the antigen it recognizes and is critical for the initiation of immune responses.
- Naive B and T cells migrate preferentially to secondary lymphoid organs. In lymph nodes and Peyer's patches, this process is mediated by binding of L-selectin on lymphocytes to peripheral lymph node addressin on high endothelial venules in lymph nodes and by binding of the CCR7 receptor on lymphocytes to the chemokines CCL19 and CCL21, which are produced in lymph nodes. There are no HEVs in splenic white pulp, and naive T and B cell migration into the spleen is not well understood.

- Within the T cell zones of the lymph nodes and spleen, naive T cells constantly move along an FRC network, interacting with DCs bound to the FRCs. If a naive T cell interacts with a DC displaying the antigen it can recognize, the T cell becomes activated to generate effector and memory T cells. If a naive T cell does not find its antigen within several hours, it will leave the lymph node via efferent lymphatics by a process dependent on the S1PR on the lymphocytes and a gradient of S1P.
- Naive B cells that enter secondary lymphoid tissues migrate into follicles in response to a gradient of the chemokine CXCL13 chemokine binding to the CXCR5 receptors on the B cell. Within the follicle, B cells move on a reticular network made of follicular dendritic cells and may bind antigens displayed by other cell types in the follicle.
- The effector and memory lymphocytes that are generated by antigen stimulation of naive cells exit the lymph node by the S1P pathway. Effector T cells have decreased expression of L-selectin and CCR7 but increased expression of integrins and E-selectin and P-selectin ligands, and these molecules mediate binding to endothelium at peripheral inflammatory sites. Effector and memory lymphocytes also express receptors for chemokines that are produced in infected peripheral tissues
- Naive follicular B cells that are activated by antigen in lymph node, spleen, or mucosal-associated lymphoid tissue may differentiate into short-lived antibody-secreting plasma cells that may stay in the secondary lymphoid organs. With help from T cells, some B cells may differentiate into plasmablasts that migrate through the blood to bone marrow or mucosal sites, where they differentiate into long-lived plasma cells that secrete antibodies for long periods.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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Chapter 4: Innate Immunity

- Overview of Innate Immunity,
  - Functions of Innate Immune Responses,
  - Comparative Features of Innate and Adaptive Immunity,
  - Evolution of Innate Immunity,
- Recognition of Microbes and Damaged Tissue by the Innate Immune System,
- Cellular Pattern Recognition Receptors,
  - Toll-Like Receptors,
  - Cytosolic Receptors for Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns,
  - Other Cell-Associated Pattern Recognition Receptors,
- Cellular Components of the Innate Immune System,
  - Epithelial Barriers,
  - Phagocytes,

    - Dendritic Cells,
    - Cytokine-Producing Innate Lymphoid Cells,
    - Natural Killer Cells,
    - T and B Lymphocytes With Limited Antigen Receptor Diversity,
    - Mast Cells,

- Soluble Effector Molecules of Innate Immunity,
    - The Complement System,
    - Pentraxins,
    - Collectins and Ficolins,

- The Inflammatory Response,
    - The Major Proinflammatory Cytokines of Innate Immunity,
    - Sequence of Events in Inflammation: Vascular Changes and Leukocyte Migration Into Tissues,
    - Ingestion and Killing of Microbes by Activated Phagocytes,
    - Role of Macrophages in Tissue Repair,
    - Systemic and Pathologic Consequences of Inflammation,

- The Antiviral Response,

- Stimulation of Adaptive Immunity,

- Mechanisms that Limit Innate Immune Responses,

- Summary,

Overview of Innate Immunity

The term **innate immunity** refers to defense mechanisms that are always present, ready to combat microbes and other offending agents. The innate immune system, which was introduced in

Chapter 1, consists of many types of cells and soluble molecules in tissues and blood that prevent microbes from invading and establishing infections. If microbes do establish a foothold, innate immune responses provide early defense, before adaptive immune responses can develop (see Fig. 1.1). In this chapter, we will describe in more detail the components, specificity, and antimicrobial mechanisms of innate immunity. The focus of much of the subsequent chapters is on the role of the adaptive immune response in host defense and disease.

Functions of Innate Immune Responses

Innate immunity is the first line of defense against infections and serves several essential functions that protect us against microbes and tissue injury. The major components of the innate immune system are barrier epithelia, which block the entry of microbes; tissue-resident sentinel cells, including macrophages, mast cells, and dendritic cells (DCs), which detect microbes that have breached epithelia and initiate host responses; white blood cells (leukocytes), including neutrophils, monocytes that become macrophages in tissues, natural killer (NK) cells, and other cells, which enter the tissues from the blood and eliminate microbes that have invaded through epithelia and also remove damaged host cells; and several types of plasma proteins that combat microbes within and outside the circulation. We discuss the functions of each of these later in the chapter. Many other cell types, including epithelial cells and other tissue cells, also possess intrinsic mechanisms for defending themselves against microbes.

The two major types of protective reactions of the innate immune system are inflammation and antiviral defense. Inflammation is the process by which circulating leukocytes and plasma proteins are brought into sites of infection in tissues and are activated to destroy and eliminate the offending agents. Inflammation is also the major reaction to damaged or dead cells unrelated to infection and to accumulations of abnormal substances in cells and tissues. Antiviral defense mechanisms prevent virus replication and promote killing of infected cells, thus eliminating reservoirs of viral infection without an inflammatory reaction (although inflammation also may contribute to defense against viruses).

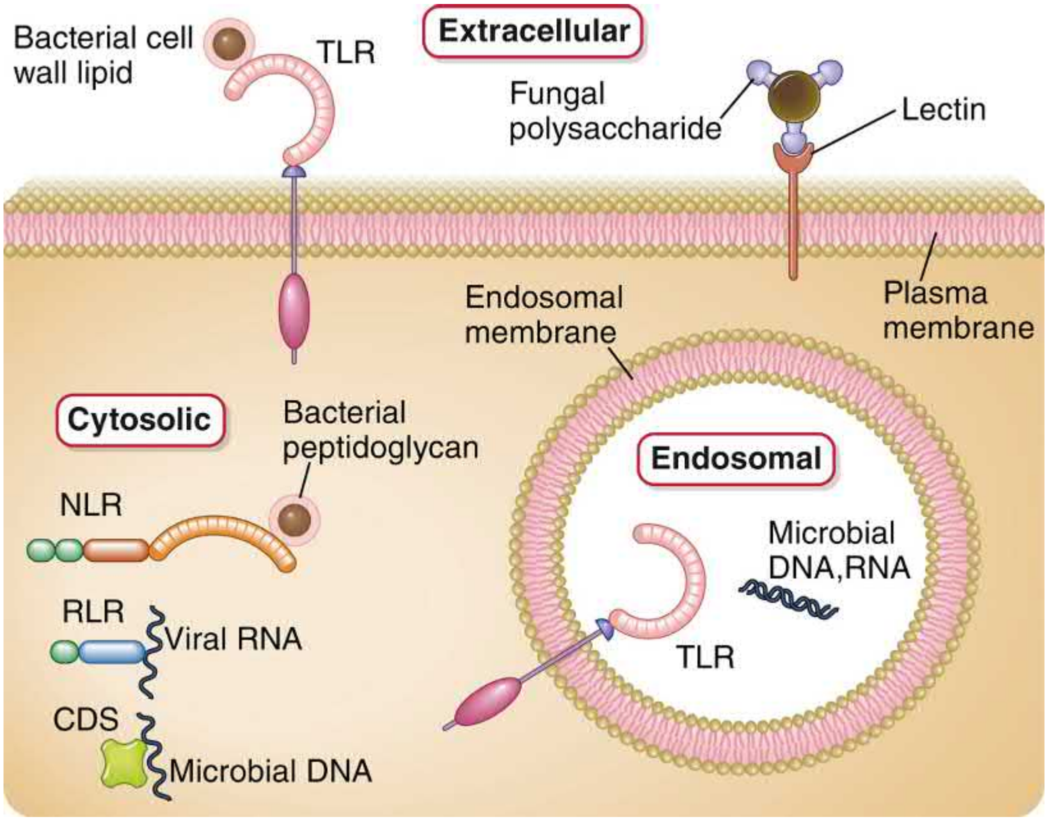

Bacterial cell wall lipid
TLR
Extracellular
Fungal polysaccharide
Lectin
Endosomal membrane
Plasma membrane
Cytosolic
Bacterial peptidoglycan
Endosomal
NLR
Microbial DNA,RNA
RLR
Viral RNA
TLR
CDS
Microbial DNA

FIGURE 4.1 Cellular locations of pattern recognition receptors of the innate immune system.Some pattern recognition molecules, including members of the TLR family (see Fig. 4.2) and lectin-like receptors, are expressed on the cell surface, where they may bind extracellular pathogen-associated molecular patterns. Other TLRs are expressed on endosomal membranes and recognize nucleic acids of microbes that have been phagocytosed by cells. Cells also contain cytosolic sensors of microbial infection, including the NLRs, RLRs, and CDSs. Only selected examples of microbial PAMPs recognized by these receptors are shown. Cytosolic receptors that recognize products of damaged cells (DAMPs) as well as some microbes are shown in Fig. 4.4. *CDS,* Cytosolic DNA sensor; *NLR,* NOD-like receptor; *RLR,* RIG-like receptor; *TLR,* Toll-like receptor.

The functions of innate immune responses have some important general features.

- ***Physical and chemical defenses at epithelial barriers, such as the skin and lining of the gastrointestinal and respiratory tracts, block microbial entry.*** Microbes are able to colonize tissues only if they are capable of crossing epithelia. If these barriers are damaged or microbes are able to penetrate them, innate and adaptive immune responses are activated to provide the next lines of defense.
- ***Innate immune responses are the initial reactions to microbes that serve to control or eliminate infection of the host by many pathogens.*** These responses are activated by microbes that traverse epithelial barriers. The responses are mediated by tissue-resident cells and other cells and plasma proteins recruited from the blood in the process of inflammation. The importance of innate immunity in host defense is illustrated by clinical observations and experimental studies showing that deficiencies, inhibition, or elimination of any of several mechanisms of innate immunity increase susceptibility to infections, even when the adaptive immune system is intact and functional. Many pathogenic microbes have evolved strategies to resist innate immunity, and these strategies are crucial for the virulence of the microbes. Innate immune responses to such microbes may keep the infection in check until adaptive immune responses are activated. Adaptive immune responses typically are more potent and specialized and therefore able to eliminate microbes that resist the defense mechanisms of innate immunity.
- ***Innate immunity eliminates damaged cells and initiates the process of tissue repair.*** These functions involve recognition and response to host molecules that are produced by,

released from, or accumulate in stressed, damaged, and dead host cells. The injury that elicits these innate responses may occur as a result of infection, or it may be sterile cell and tissue damage in the absence of infection.

- ***Innate immune responses stimulate adaptive immune responses and can influence the nature of the adaptive responses to make them optimally effective against different types of microbes.*** Thus, innate immunity not only serves as the initial line of defense early after infection but also provides the danger signals that alert the adaptive immune system to respond. Moreover, different components of the innate immune system often react in distinct ways to different microbes (e.g., extracellular bacteria versus intracellular viruses) and thereby influence the type of adaptive immune response that develops. We will return to this concept at the end of the chapter.

Comparative Features of Innate and Adaptive Immunity

In order to understand how innate and adaptive immunity complement each other to protect against pathogens, it is instructive to highlight their important differences.

- Innate immune responses to a microbe develop rapidly and do not require prior exposure to the microbe. In other words, innate immune effector cells and molecules are present in sufficient quantities even before infection and are fully functional or quickly become activated by microbes to prevent, control, or eliminate infections. In contrast, effective adaptive immune responses to a newly introduced microbe develop over several days as clones of naive antigen-specific lymphocytes expand and differentiate into functional effector cells.

- For most innate responses to microbes, there is no appreciable change in the quality or magnitude of the response upon repeated exposure—that is, there is little or no memory. In contrast, repeated exposure to a microbe enhances the rapidity, magnitude, and effectiveness of adaptive immune responses. There is emerging evidence for some memory in innate immunity, in that macrophage and NK cell responses to certain infections are increased in magnitude upon subsequent infections. It is not clear how specific these memory-like innate responses are, which and how many microbes are capable of eliciting them, or if such responses contribute to increased protection against repeat infections.
- The innate and adaptive immune systems differ greatly in specificity for microbial structures and in the diversity of their receptors, as discussed later.

Evolution of Innate Immunity

Innate immunity is phylogenetically the oldest part of the immune system. It co-evolved with microbes to protect all organisms from infections. Some components of the mammalian innate immune system are remarkably similar to components in plants and insects, suggesting that these appeared in common ancestors long ago in evolution. For example, peptides that are toxic to bacteria and fungi, called defensins, are found in plants and mammals and have essentially the same tertiary structure in both life forms. A family of receptors that we will discuss in detail later in this chapter, called Toll-like receptors, recognize pathogenic microbes and activate antimicrobial defense mechanisms. Toll-like receptors are found in every life form in the evolutionary tree from insects up to mammals. The major signal transduction pathway that Toll-like receptors engage to activate cells, called the NF-κB (nuclear factor kappa B) pathway in mammals, also shows remarkable evolutionary conservation. In fact, most of the mechanisms of innate immune defense that we will discuss in this chapter appeared very early in evolution, when the first multicellular organisms evolved, about 750 million years ago. An adaptive immune system, in contrast, is clearly recognizable only in vertebrates that developed about 350 to 500 million years ago. A testament to the importance of innate immunity is that the human genome contains approximately 850 genes that are directly related to innate immune responses, compared to about 575 for adaptive immunity.

We begin our discussion of the innate immune system by describing how it recognizes microbes and damaged host cells. We will then proceed to the individual components of innate immunity and their functions in host defense.

Recognition of Microbes and Damaged Tissue by the Innate Immune System

The specificities of innate immune recognition have evolved to combat microbes and are different from the specificities of the adaptive immune system in several respects (Table 4.1).

- The innate immune response is activated by recognition of a relatively limited set of molecular structures that either are products of microbes or are expressed by injured or dead host cells. It is estimated that the innate immune system recognizes only about 1000 products of microbes and damaged cells. By contrast, the adaptive immune system potentially can recognize millions of different microbial antigens and can also recognize nonmicrobial environmental antigens and the self antigens that are normally present in healthy tissues.
- The innate immune system uses germline-encoded invariant receptors to recognize microbial and other products. By contrast, the adaptive immune system uses highly variable and diverse receptors to recognize foreign antigens. The specificity and receptors of innate immunity are described later.

The innate immune system recognizes molecular structures that are produced by microbial pathogens. The microbial substances that stimulate innate immunity are often shared by classes of microbes and are called **pathogen-associated molecular patterns (PAMPs).** Different types of microbes (e.g., viruses, gram-negative bacteria, gram-positive bacteria, fungi) express different PAMPs (Table 4.2). These structures include nucleic acids that are unique to or more abundant in microbes than in host cells, such as double-stranded RNA found in replicating viruses and unmethylated CpG DNA sequences found in bacteria; features of proteins that are found in microbes, such as initiation by *N*-formylmethionine, which is typical of bacterial proteins; and complex lipids and carbohydrates that are synthesized by microbes but not by mammalian cells, such as lipopolysaccharide (LPS) in gram-negative bacteria, lipoteichoic acid in gram-positive bacteria, and oligosaccharides with terminal mannose residues found in microbial but not in mammalian glycoproteins.

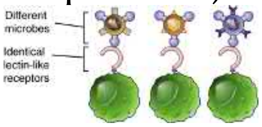
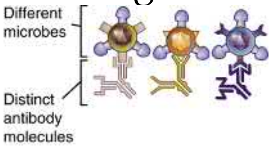
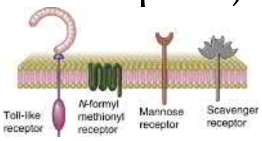
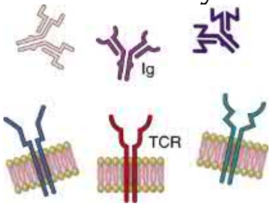
The innate immune system detects the presence of infection but not the specific pathogens. By recognizing PAMPs, which are produced by broad classes of microbes, and products of cell damage that is often induced by pathogens, the innate immune system triggers host defense regardless of the particular species of microbe. In contrast, the adaptive immune system is capable of recognizing many more and diverse foreign substances (antigens), which may be unique to different individual microbial species or may be unrelated to microbial infection or tissue injury.

The innate immune system recognizes microbial products that are often essential for survival of the microbes. This evolutionary adaptation of innate immune recognition is important because it ensures that microbes cannot evade innate immunity by mutating the molecules recognized by the host. An example of a target of innate immunity that is indispensable for microbes is double-stranded viral RNA, which is an essential intermediate in the life cycle of many viruses. Similarly, LPS and lipoteichoic acid are structural components of bacterial cell walls that are recognized by innate immune receptors; both are required for bacterial survival.

In contrast, as we will see in Chapter 16, microbes may mutate or lose many of the antigens that are recognized by the adaptive immune system, thereby evading host defense without compromising their own survival.

TABLE 4.1

Specificity of Innate and Adaptive Immunity

	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns)  Different microbes Identical lectin-like receptors	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens  Different microbes Distinct antibody molecules
Number of microbial molecules recognized	About 1000 molecular patterns (estimated)	>10^7 antigens
Receptors	Encoded in germline; limited diversity (pattern recognition receptors)  Toll-like receptor N-formyl methionyl receptor Mannose receptor Scavenger receptor	Encoded by genes produced by somatic recombination of gene segments; greater diversity 

	Innate Immunity	**Adaptive Immunity**
Number and types of receptors	<100 different types of invariant receptors	Only two types of receptors (Ig and TCR), with millions of variations of each
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Genes encoding receptors	Germline encoded, in all cells	Formed by somatic recombination of gene segments only in B and T cells
Discrimination of self and nonself	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

Ig, Immunoglobulin; *TCR,* T cell antigen receptor.

The innate immune system also recognizes endogenous molecules that are produced by or released from damaged and dying cells. These substances are called **damage-associated molecular patterns (DAMPs)** (see Table 4.2). DAMPs may be produced as a result of cell damage caused by infections, but they may also indicate sterile injury to cells caused by any of many insults, such as chemical toxins, burns, trauma, or loss of blood supply. DAMPs are generally not released from cells dying by apoptosis. In some cases, endogenous molecules, including some cytokines, that are produced by healthy cells are released when the cells are damaged or die, and they then stimulate innate responses. These molecules are a subset of DAMPs and are sometimes called alarmins because their presence outside cells alarms the immune system that something is causing cell damage or death.

The innate immune system uses several types of cellular receptors, present in different locations in cells, and soluble molecules in the blood and mucosal secretions to recognize PAMPs and DAMPs (Table 4.3) **.** Cell-associated recognition molecules of the innate immune system are expressed by phagocytes (primarily macrophages and neutrophils), DCs, epithelial cells that form the barrier interface between the body and the external environment, mast cells, and many other types of cells that reside in tissues. The cellular receptors for pathogen- and damage-associated molecular patterns are called **pattern recognition receptors.** They are expressed on the surface, in phagocytic vesicles, and in the cytosol of various cell types, all of which are locations where microbes or their products may be present (see Fig. 4.1). When these cell-associated pattern recognition receptors bind to PAMPs and DAMPs, they activate signal transduction pathways that promote the antimicrobial and proinflammatory functions of the cells in which they are expressed. In addition, many proteins present in the blood and extracellular fluids recognize PAMPs (see Table 4.3). These soluble molecules are responsible for facilitating the clearance of microbes from the blood and extracellular fluids by enhancing uptake into phagocytes or by activating extracellular killing mechanisms.

TABLE 4.2

Pathogen-Associated Molecular Patterns	
Nucleic acids	ssRNA: viruses
	dsRNA: viruses
	Unmethylated CpG: viruses, bacteria
Proteins	Pilin: bacteria
	Flagellin: bacteria
Cell wall lipids	LPS: gram-negative bacteria
	Lipoteichoic acid: gram-positive bacteria
Carbohydrates	Mannan: fungi, bacteria
	Glucans: fungi
Damage-Associated Molecular Patterns	
Stress-induced proteins	HSPs
Crystals	Monosodium urate
Proteolytically cleaved extracellular matrix	Proteoglycan peptides
Mitochondrial components found outside mitochondria	Extracellular formylated peptides and ATP
Nuclear proteins or nucleic acids found outside nucleus	Extracellular HMGB1, histones, cytoplasmic dsDNA

ATP, Adenosine triphosphate; *CpG,* cytosine-guanine–rich oligonucleotide; *dsRNA,* double-stranded RNA; *HMGB1,* high-mobility group box 1; *HSP,* heat shock protein; *LPS,* lipopolysaccharide; *ssRNA,* single-stranded RNA.

The receptors of the innate immune system are encoded by inherited (germline) genes, whereas the genes encoding receptors of adaptive immunity are generated by somatic recombination of gene segments in the precursors of mature lymphocytes. As a result, the diversity of innate immune system receptors and the range of their specificities are small compared with those of B and T cells of the adaptive immune system. It is estimated that innate immune recognition is mediated by about 100 different receptors belonging to several protein families, whereas in the adaptive immune system there are only two families of receptors (immunoglobulins [Igs] and T cell receptors) each with millions of variations that recognize a vast number of antigens. Furthermore, whereas the adaptive immune system can distinguish between antigens of different microbes of the same class and even different antigens of one microbe, innate immunity can distinguish only classes of microbes, or only damaged cells from healthy cells, but not particular species of microbes or cell types.

The innate immune system does not react against normal, healthy cells and tissues. This characteristic is, of course, essential for the health of the organism. The failure to recognize healthy self is due to three main mechanisms: normal cells do not produce ligands for innate immune receptors, these receptors are located in cellular compartments where they do not encounter host molecules they could recognize, and regulatory proteins expressed by normal cells prevent activation of various components of innate immunity. We will discuss examples of such regulation later in this chapter.

With this introduction, we can proceed to a discussion of the large variety of molecules in the body that are capable of recognizing PAMPs and DAMPs, focusing on their specificity, location, and functions. We will begin with cell-associated receptors expressed on membranes or in the cytosol of cells. The soluble recognition and effector molecules of innate immunity, found in the blood and extracellular fluids, are described later.

Cellular Pattern Recognition Receptors

Most cell types express pattern recognition receptors and therefore are capable of participating in innate immune responses. Phagocytes, especially macrophages, and DCs express the widest variety and greatest number of these receptors. This is in keeping with the fundamental role of phagocytes in detecting microbes and damaged cells and ingesting them for destruction and the role of DCs in reacting to microbes in ways that elicit inflammation and subsequent adaptive immunity. Pattern recognition receptors are linked to intracellular signal transduction pathways that activate various cellular responses, including the production of molecules that promote inflammation and molecules that destroy microbes.

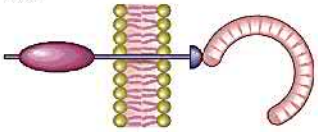
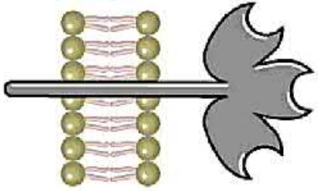
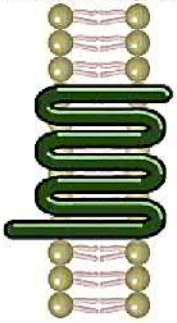
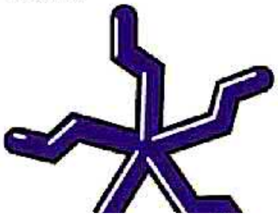
We will organize our discussion around several distinct classes of cellular pattern recognition receptors that differ in their structure and specificity for various types of microbes.

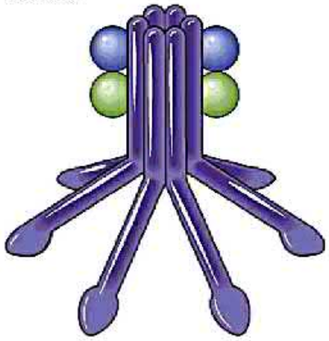
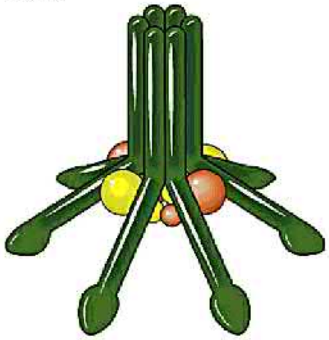
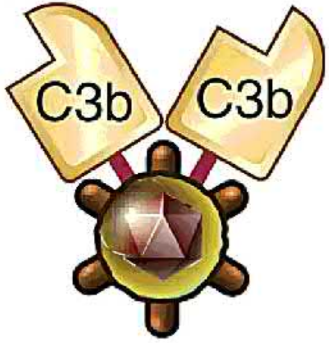
Toll-Like Receptors

Toll-like receptors (TLRs) are an evolutionarily conserved family of pattern recognition receptors expressed by many cell types that

recognize products of a wide variety of microbes, as well as molecules expressed or released by stressed and dying cells. *Toll* was discovered as a Drosophila gene involved in establishing the dorsal-ventral axis during development of the fruit fly, but subsequently it was discovered that the Toll protein also mediated antimicrobial responses in these organisms. Furthermore, the cytoplasmic domain of Toll was found to be similar to the cytoplasmic region of the receptor for the innate immune cytokine interleukin-1 (IL-1). These discoveries led to the identification of mammalian homologs of Toll, which were named Toll-like receptors. There are 10 different functional TLRs in humans, named TLR1 through TLR10 (Fig. 4.2). Mice express TLRs homologous to human TLRs 1 through 9, plus three more (TLRs 11 through 13), but they do not express TLR10. The function of TLR10 remains poorly understood compared to those of other TLRs.

TABLE 4.3

Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Cell-Associated			
TLRs 	Plasma membrane and endosomal membranes of DCs, phagocytes, B cells, endothelial cells, and many other cell types	TLRs 1–9	Various microbial molecules including bacterial LPS, peptidoglycans, viral nucleic acids
NLRs	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2	Bacterial cell wall peptidoglycans
		NLRP family (inflammasomes)	Intracellular crystals (urate, silica); changes in cytosolic ATP and ion concentrations; lysosomal damage
RLRs	Cytosol of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
CDSs	Cytosol of many cell types	AIM2; STING-associated CDSs	Bacterial and viral DNA
CLRs	Plasma membranes of phagocytes	Mannose receptor, DC-SIGN	Microbial surface carbohydrates with terminal mannose and fucose
		Dectin-1, Dectin-2	Glucans present in fungal and bacterial cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
***N*-Formyl met-leu-phe receptors** 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues
Soluble			
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine

Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Collectins 	Plasma	Mannose-binding lectin	Carbohydrates with terminal mannose and fucose
	Alveoli	Surfactant proteins SP-A and SP-D	Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-Acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	Various complement proteins	Microbial surfaces

AIM2, Absent in melanoma-2; *CDSs,* cytosolic DNA sensors; *CLRs,* C-type lectin–like receptors; *DAMP,* damage-associated molecular pattern; *DC,* dendritic cells; *MDA,* melanoma differentiation-associated gene; *NLRs,* NOD-like receptors; *NOD,* nucleotide oligomerization domain; *PAMP,* pathogen-associated molecular pattern; *RLRs,* RIG-like receptors; *SP-D,* surfactant protein D; *STING,* stimulator of IFN (interferon) genes; *TLRs,* Toll-like receptors.

The TLRs are integral membrane glycoproteins that contain leucine-rich repeats flanked by characteristic cysteine-rich motifs in their extracellular regions, which are involved in ligand binding, and a Toll/IL-1 receptor (TIR) domain in their cytoplasmic tails, which is essential for signaling. TIR domains are also found in the cytoplasmic tails of the receptors for the cytokines IL-1, IL-18, and IL-33, and similar signaling pathways are engaged by TLRs and these cytokines.

TLRs are involved in responses to a wide variety of molecules that are expressed by microbes but not by healthy mammalian cells. The ligands that the different TLRs recognize are structurally diverse and include products of all classes of microorganisms (see Fig. 4.2), as in the following examples:

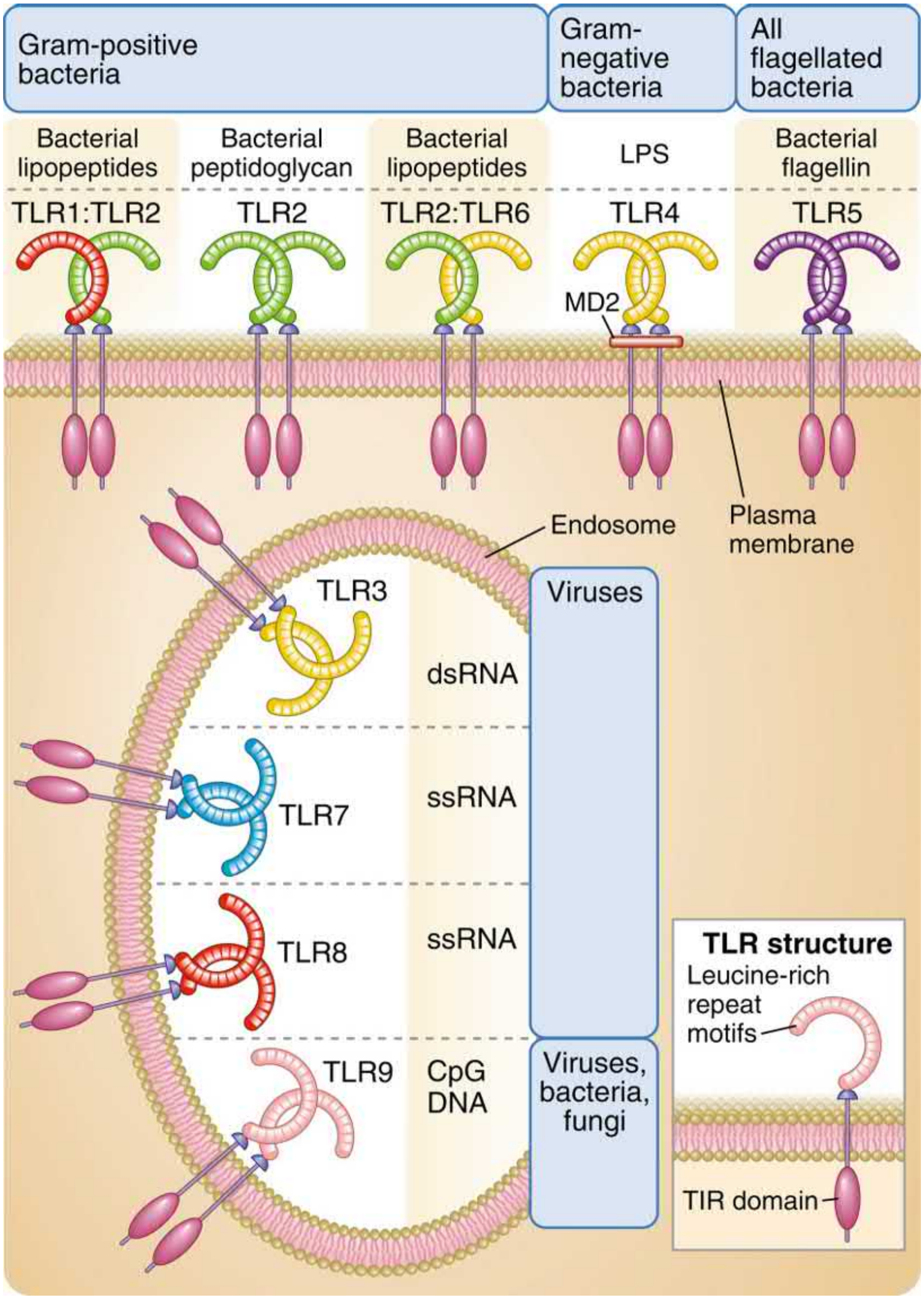

Gram-positive bacteria
Gram-negative bacteria
All flagellated bacteria
Bacterial lipopeptides
Bacterial peptidoglycan
Bacterial lipopeptides
LPS
Bacterial flagellin
TLR1:TLR2
TLR2
TLR2:TLR6
TLR4
TLR5
MD2
Endosome
Plasma membrane
TLR3
dsRNA
Viruses
TLR7
ssRNA
TLR8
ssRNA
TLR9
CpG DNA
Viruses, bacteria, fungi
TLR structure
Leucine-rich repeat motifs
TIR domain

FIGURE 4.2 Structure, location, and specificities of mammalian Toll-like receptors.Note that some TLRs are expressed on the cell surface and others in endosomes. TLRs may form homodimers or heterodimers. *dsRNA,* Double-stranded RNA; *LPS,* lipopolysaccharide; *ssRNA,* single-stranded RNA; *TIR,* Toll IL-1 receptor; *TLR,* Toll-like receptor.

- *Bacterial cell wall constituents:* LPS of gram-negative bacteria, which binds TLR4; and peptidoglycan and lipoteichoic acid of gram-positive bacteria, which bind TLR2.
- *Bacterial surface proteins:* flagellin, a protein subunit component of the flagella of motile bacteria, which binds to TLR5
- *Viral nucleic acids:* double-stranded RNAs that make up the genomes of some viruses, are generated during the life cycle of most viruses, and bind TLR3; single-stranded RNAs, which are distinguished from cellular cytoplasmic single-stranded RNA transcripts by their location within endosomes and by their high guanosine and uridine content, bind TLR7 and TLR8; and unmethylated CpG nucleotides, which are common in prokaryotic DNA but rare in vertebrate genomes, bind TLR9.

TLRs are also involved in responses to endogenous molecules whose expression or location indicates cell damage. Examples of host molecules that engage TLRs include heat shock proteins (HSPs), which are chaperones induced in response to various cell stresses and bind to TLR4, and high-mobility group box 1 (HMGB1), an abundant DNA-binding protein involved in transcription and DNA repair, which binds to TLR2. Both HSPs and HMGB1 are normally intracellular proteins but may become extracellular when released from injured or dying cells. From their extracellular location, they activate TLR2 and TLR4 signaling in DCs, macrophages, and other cell types.

The structural basis of TLR specificities resides in the multiple extracellular or endosomal lumenal leucine-rich modules of these receptors, which bind directly to PAMPs or to adaptor molecules that bind the PAMPs. There are between 16 and 28 leucine-rich repeats in TLRs. Each of these modules is composed of 20 to 30 amino acids that include conserved LxxLxLxxN motifs (where L is leucine, x is any amino acid, and N is asparagine) and amino acid residues that vary among TLRs. The ligand-binding variable residues of the modules are on the convex surface formed by α helices and β turns or loops. These repeats contribute to the ability of some TLRs to bind hydrophobic molecules such as bacterial LPS. Ligand binding to the leucine-rich domains induces physical interactions between TLR molecules and the formation of TLR dimers. The ability of TLRs to form homodimers or heterodimers with different specificities increases the number of PAMPs that can be recognized by the small number of TLRs expressed. For example, TLR1/TLR2 dimers recognize different lipopeptides than TLR2/TLR6 dimers, and TLR2/TLR2 homodimers recognize peptidoglycan.

The binding of TLRs to their ligands is also influenced by various non-TLR accessory molecules. This is best defined for the TLR4 response to LPS. LPS first binds to soluble LPS-binding protein in the blood or extracellular fluid, and this complex facilitates delivery of the LPS to the surface of the responding cell. An extracellular protein called MD2 (myeloid differentiation protein 2) binds to the lipid A component of LPS, forming a complex that then interacts with TLR4 and initiates signaling. Another protein, called CD14, is also required for efficient LPS-induced signaling. CD14 is expressed by most cells (except endothelial cells) as a soluble protein or as a glycophosphatidylinositol-linked membrane protein.

TLRs are found on the cell surface and on intracellular membranes and are thus able to recognize microbes in different cellular locations (see Fig. 4.2) **.** TLRs 1, 2, 4, 5, and 6 are expressed on the plasma membrane, where they recognize various bacterial and fungal PAMPs in the extracellular environment. Some of the most potent microbial stimuli for innate immune responses bind to these plasma membrane TLRs, such as bacterial LPS and lipoteichoic acid, which are recognized by TLR4 and TLR2, respectively. In contrast, TLRs 3, 7, 8, and 9 are mainly expressed inside cells on endosomal membranes, where TLR3 detects double-stranded RNA, TLR7 and TLR8 detect single-stranded RNA produced by viruses, and TLR9 detects unmethylated CpG motifs in bacterial or viral DNA. Single- and double-stranded RNA are not unique to microbes, but their location in endosomes likely reflects origin from microbes. This is because host cell RNA is not normally present in endosomes, but microbial RNA may end up in endosomes of neutrophils, macrophages, or DCs when the microbes are phagocytosed by these cells. Enzymatic digestion of the microbes within endosomes will release their nucleic acids so these are able to bind TLRs in the endosomal membrane. Thus, the endosomal TLRs may distinguish nucleic acids of normal cells from microbial nucleic acids on the basis of the cellular location of these molecules. A protein called UNC93B is required for the transfer of TLRs synthesized in the endoplasmic reticulum to their endosomal localization. Genetic deficiency in UNC93B or TLR3 leads to susceptibility to certain viral infections, especially herpes simplex virus encephalitis, demonstrating the importance of the endosomal location of TLRs for innate defense against viruses.

TLR recognition of microbial ligands results in the activation of several signaling pathways and ultimately transcription factors, which induce the expression of genes whose products are important for inflammatory and antiviral responses (Fig. 4.3) **.** The signaling pathways are initiated by ligand binding to the TLR at the cell surface or in endosomes, leading to dimerization of the TLR proteins. Ligand-induced TLR dimerization is predicted to bring the TIR domains of the cytoplasmic tails of each protein close to one another. This is followed by recruitment of TIR domain–containing adaptor proteins, which facilitate the recruitment and activation of various protein kinases, leading to the activation of different transcription factors. The major transcription factors that are activated by TLR signaling pathways are NF-κB, interferon response factor 3 (IRF3), and IRF7. NF-κB stimulates the expression of genes encoding many of the molecules required for inflammatory responses, including inflammatory cytokines (e.g., tumor necrosis factor [TNF] and IL-1), chemokines (e.g., CCL2 and CXCL8), and endothelial adhesion molecules (e.g., E-selectin). IRF3 and IRF7 promote expression of the genes encoding interferon (IFN)-α and IFN-β, respectively, which are both type I IFNs that are important for antiviral innate immune responses.

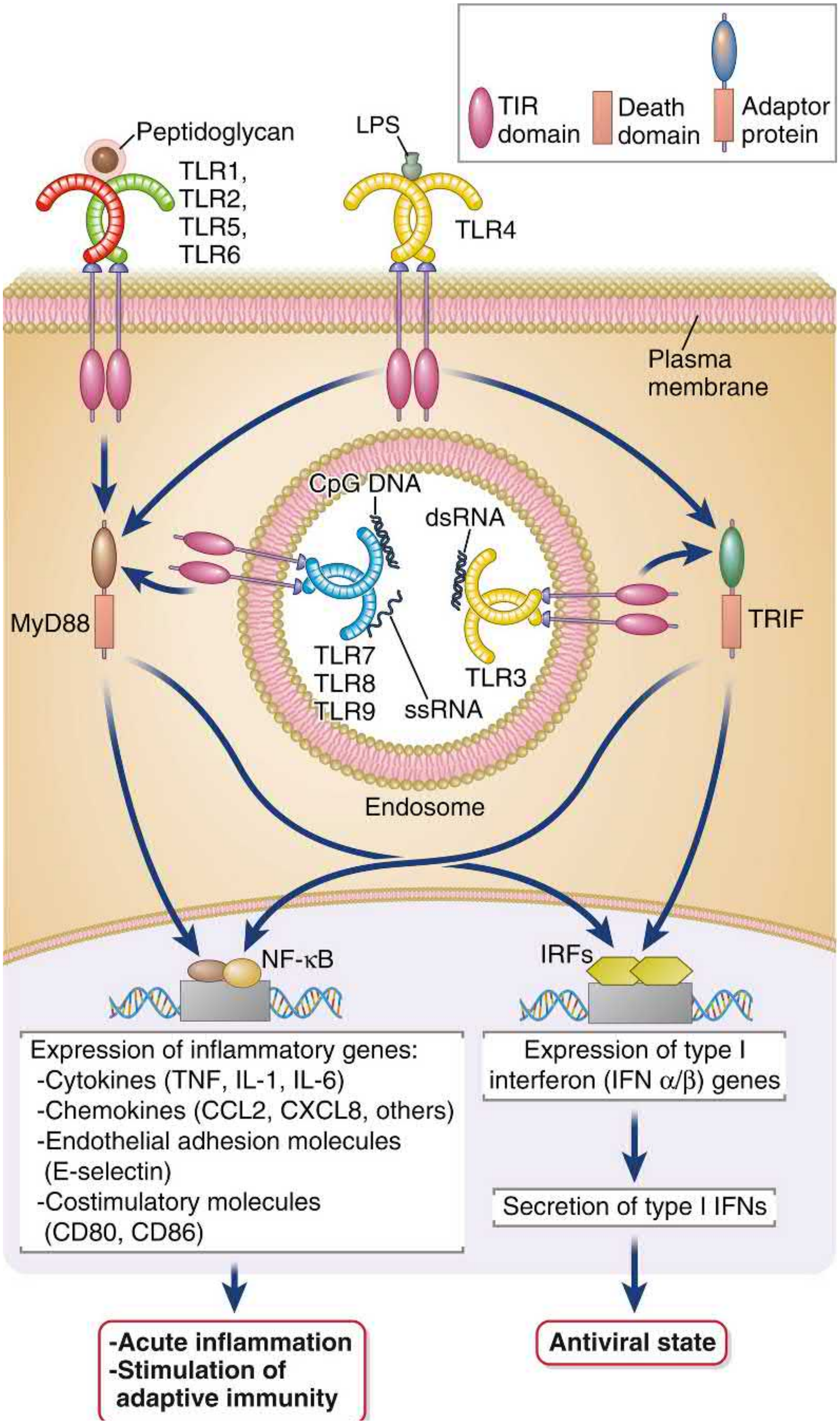

TIR domain
Death domain
Adaptor protein
Peptidoglycan
TLR1,
TLR2,
TLR5,
TLR6
LPS
TLR4
Plasma membrane
CpG DNA
dsRNA
MyD88
TRIF
TLR7
TLR8
TLR9
ssRNA
TLR3
Endosome
NF-κB
IRFs
Expression of inflammatory genes:
-Cytokines (TNF, IL-1, IL-6)
-Chemokines (CCL2, CXCL8, others)
-Endothelial adhesion molecules (E-selectin)
-Costimulatory molecules (CD80, CD86)
Expression of type I interferon (IFN α/β) genes
Secretion of type I IFNs
-Acute inflammation
-Stimulation of adaptive immunity
Antiviral state

FIGURE 4.3 Signaling pathways and functions of Toll-like receptors. TLRs 1, 2, 5, and 6 use the adaptor protein MyD88 and activate the transcription factor NF-κB, which induces inflammatory gene expression. TLR3 uses the adaptor protein TRIF, which activates the IRF3 transcription factor and promotes IFN-β expression and NF-κB. TLR4 uses both MyD88 and TRIF, leading to activation of NF-κB and IRF3 pathways, respectively. TLRs 7, 8, and 9 in the endosome use MyD88, leading to activation of both NF-κB and IRF7, promoting expression of genes whose products mediate inflammation and antiviral defense. *dsRNA,* Double-stranded RNA; *IFN,* interferon; *IRFs,* interferon regulatory factors; *NF-κB,* nuclear factor kappa B; *ssRNA,* single-stranded DNA; *TIR,* Toll IL-1 receptor; *TLR,* Toll-like receptors; *TRIF,* TIR-domain-containing adapter-inducing interferon-β.

Different combinations of adaptors and signaling intermediates are used by different TLRs, accounting for the common and unique downstream effects of the TLRs. The cell surface TLRs 1, 5, and 6 engage the adaptor MyD88, leading mainly to NF-κB activation and inflammatory responses but not robust IRF activation or type I IFN responses. The endosomal TLRs 7, 8, and 9 also engage MyD88, but in these cases, downstream signaling leads to both NF-κB and IRF7 activation. NF-κB promotes inflammatory responses, and IRF7 promotes IFN-*α* expression that mediates antiviral responses. Cell surface TLR4 engages both MyD88 and the adaptor called TRIF (TIR domain–containing adaptor inducing IFN-β), both of which induce NF-κB activation, whereas TRIF alone induces IRF3 activation that promotes IFN-β expression. Endosomal TLR3 signals only through TRIF, which mediates both NF-κB and IRF3 activation.

Thus, TLR4 and TLR3 signaling induce both inflammatory and IFN-β–mediated responses.

Cytosolic Receptors for Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns

In addition to the membrane-bound TLRs, which sense pathogens outside cells or in endosomes, the innate immune system has evolved to equip cells with pattern recognition receptors that detect infection or cell damage in the cytosol (see Fig. 4.1 and Table 4.3). Three major classes of these cytosolic receptors are NOD-like receptors, retinoic acid–inducible gene (RIG)-like receptors, and cytosolic DNA sensors. These cytosolic receptors, similar to the TLRs, are linked to signal transduction pathways that promote inflammation or type I IFN production. Some of the cytosolic sensors induce the formation of enzyme complexes called inflammasomes, which proteolytically generate a biologically active inflammatory cytokine IL-1β from an inactive precursor and also may induce cell death. The ability of the innate immune system to detect infection in the cytosol is important because parts of the normal life cycles of some microbes, such as viral gene translation and viral particle assembly, take place in the cytosol. Some bacteria and parasites have mechanisms that enable them to escape from phagocytic vesicles into the cytosol. Microbes can produce toxins that create pores in host cell plasma membranes, including endosomal membranes, through which microbial molecules can enter the cytosol. These pores also can result in changes in the concentration of endogenous molecules, such as ions, in the cytoplasm, which are reliable signs of infection and damage and are detected by the cytosolic receptors.

NOD-Like Receptors: NOD1 and NOD2

NOD-like receptors (NLRs) are a family of more than 20 different cytosolic proteins, some of which recognize PAMPs and DAMPs and

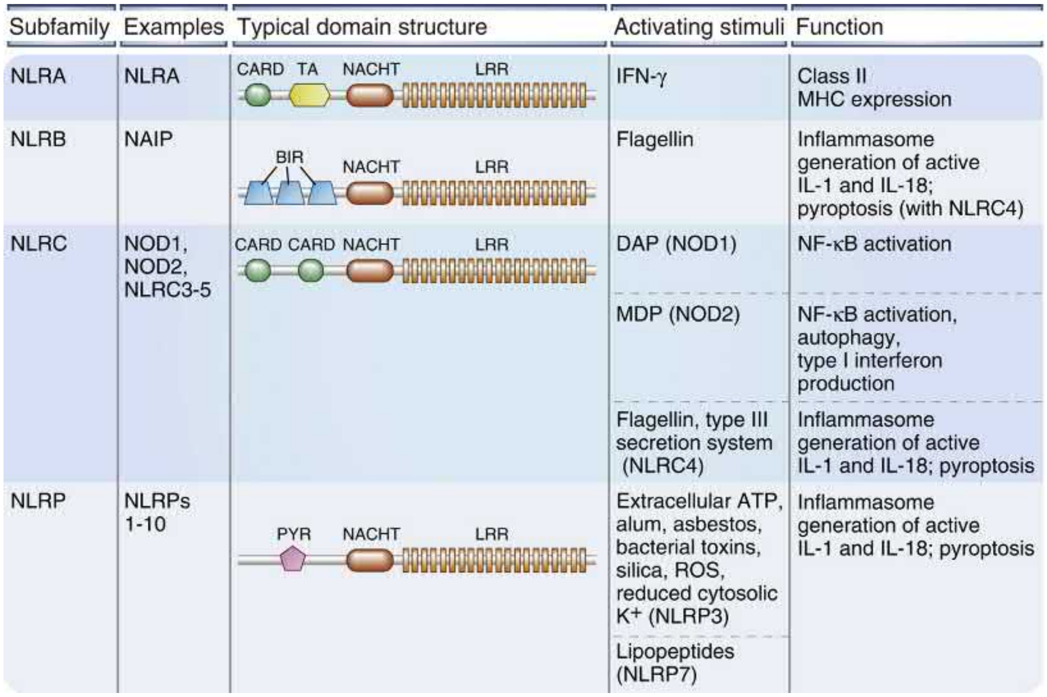
recruit other proteins to form signaling complexes that promote inflammation. Typical NLRs include a C-terminal leucine-rich repeat domain that senses the presence of ligand; a central NOD (nucleotide oligomerization domain, also called NACHT) domain required for NLR proteins to bind to one another and form oligomers; and an N-terminal effector domain, which recruits other proteins to form signaling complexes (Fig. 4.4). There are three NLR subfamilies that serve as innate immune receptors, each using a different effector domain to initiate signaling. These include NLRB, which uses the BIR (baculovirus inhibition of apoptosis protein repeat) effector domain; NLRC, which uses CARDs (caspase recruitment and activation domains); and NLRP, which uses pyrin domains (so called because they are found in proteins involved in causing fever) (see Fig. 4.4). NLRs are found in a wide variety of cell types, although some have restricted cellular expression. Some of the best studied NLRs are found in immune and inflammatory cells and in barrier epithelial cells. We will discuss two NLR sensors of bacterial PAMPs here, named NOD1 and NOD2, and other NLRs later, when we discuss inflammasomes.

NOD1 and NOD2, members of the NLRC subfamily, are expressed in the cytosol of several cell types, including mucosal epithelial cells and phagocytes, and they respond to bacterial cell wall peptidoglycans. NOD2 is highly expressed in intestinal Paneth cells in the small bowel, where it stimulates expression of antimicrobial substances called defensins in response to pathogens. NOD1 recognizes a glycosylated tripeptide called diaminopimelic acid (DAP), derived mainly from gram-negative bacterial peptidoglycan, whereas NOD2 recognizes a distinct molecule called muramyl dipeptide (MDP), derived from both gram-negative and gram-positive peptidoglycans. These peptides are released from intracellular or extracellular bacteria; in the latter case, their presence in the cytosol requires specialized bacterial mechanisms of delivery of the peptides into host cells. These mechanisms include type III and type IV secretion systems, which have evolved in pathogenic bacteria as a means of delivering toxins into host cells. NOD1 and NOD2 also can be activated by other microbial PAMPs, including bacterial proteins and viral RNAs. When oligomers of NODs recognize their ligands, a conformational change occurs that allows the CARD effector domains of the NOD proteins to recruit multiple copies of the kinase RIP2, forming a signaling complex that has been called the NOD signalosome. The RIP2 kinases in these complexes activate NF-κB, which stimulates production of cytokines and other molecules involved in inflammation, similar to TLRs that signal through MyD88, discussed earlier. Both NOD1 and NOD2 appear to be important in innate immune responses to bacterial pathogens in the gastrointestinal tract, such as *Helicobacter pylori* and *Listeria monocytogenes*.

Certain *NOD2* gene polymorphisms increase the risk for an inflammatory disease of the bowel called Crohn's disease. A possible explanation for this association is that the disease-associated NOD2 variants are defective in their ability to sense microbial products, resulting in defective innate responses against commensal and pathogenic organisms in the intestine. If these organisms gain access to the intestinal wall, they may trigger chronic inflammation. Also, gain-of-function mutations of NOD2 that cause increased NOD signaling and NF-κB activation lead to a systemic inflammatory disease called Blau syndrome.

Cytosolic DNA Sensors and the STING Pathway

Cytosolic DNA sensors (CDSs) are molecules that detect double-stranded (ds) DNA in the cytosol and activate signaling pathways that initiate antimicrobial responses, including type I IFN production and autophagy. DNA may be released into the cytosol from intracellular microbes. Furthermore, host DNA damage due to various factors, such as radiation, toxins, or mutations, will lead to the delivery of that DNA into the cytoplasm in micronuclei whose nuclear envelopes degrade, exposing the DNA to cytosolic CDSs. Cytosolic DNA may also be produced during normal turnover but is usually rapidly degraded by endonucleases. The ability of the innate immune system to distinguish and react to microbial or damaged self DNA and not normal self DNA is partly related to the location of most of the DNA sensors in the cytosol, where DNA should not be in normal uninfected cells.



Subfamily	Examples	Typical domain structure	Activating stimuli	Function
NLRA	NLRA	CARD TA NACHT LRR	IFN-γ	Class II MHC expression
NLRB	NAIP	BIR NACHT LRR	Flagellin	Inflammasome generation of active IL-1 and IL-18; pyroptosis (with NLRC4)
NLRC	NOD1, NOD2, NLRC3-5	CARD CARD NACHT LRR	DAP (NOD1)	NF-κB activation
			MDP (NOD2)	NF-κB activation, autophagy, type I interferon production
			Flagellin, type III secretion system (NLRC4)	Inflammasome generation of active IL-1 and IL-18; pyroptosis
NLRP	NLRPs 1-10	PYR NACHT LRR	Extracellular ATP, alum, asbestos, bacterial toxins, silica, ROS, reduced cytosolic K$^+$ (NLRP3)	Inflammasome generation of active IL-1 and IL-18; pyroptosis
			Lipopeptides (NLRP7)	

FIGURE 4.4 NOD-like receptors.Members of the NOD-like receptor *(NLR)* family that perform immune functions can be assigned to one of four subfamilies: NLRA, NLRB, NLRC, and NLRP, each with a different N-terminal effector domain. NLRA, better known as *CIITA,* is a transcription factor that has an N-terminal transactivating *(TA)* domain required for class II major histocompatibility complex *(MHC)* gene expression. NLRB has a baculovirus inhibition of apoptosis protein repeat *(BIR)* domain, of unknown function. NLRC members have an N-terminal caspase recruitment and activation domain *(CARD),* which is involved in caspase-1 activation. NLRP members have a pyrin *(PYD)* domain, which also activates caspase-1. All NLRs contain a central NOD or NACHT (NAIP, CIITA, HET-E, and TP1) domain involved in nucleotide binding, and C-terminal leucine-rich repeat domains involved in ligand recognition. Some of the principal functions and activating ligands of NLRs are shown. *ATP,*

Adenosine triphosphate; *DAP,* diaminopimelic acid; *IFN,* interferon; *IL,* interleukin; *LRR,* leucine-rich repeat; *MDP,* muramyl dipeptide; *NF-κB,* nuclear factor kappa B; *NOD,* nucleotide oligomerization domain; *ROS,* reactive oxygen species.

The STING (stimulator of IFN genes) pathway is an important mechanism of DNA-induced activation of type I IFN responses (Fig. 4.5) **.** In this pathway, cytosolic dsDNA activates the enzyme cGAS (cyclic guanosine monophosphate–adenosine monophosphate [GMP-AMP] synthase), which generates a signaling molecule called cGAMP (cyclic GMP-AMP). STING is an endoplasmic reticulum–localized transmembrane adaptor protein whose cytosolic tail binds to cGAMP with high affinity, inducing a conformational change that leads to its translocation from the endoplasmic reticulum to the Golgi apparatus. After localization to the Golgi, STING activates the TBK1 kinase, which phosphorylates and activates the IRF3 transcription factor, leading to type I IFN gene expression. STING also responds to other cytosolic DNA sensors besides cGAS, including DAI (DNA-dependent activator of IFN-regulatory factors) and IFI16 (interferon inducible protein 16). In addition to inducing IFN production, STING stimulates autophagy, a mechanism by which cells degrade their own organelles, such as mitochondria, by sequestering them within membrane-bound vesicles and fusing the vesicles with lysosomes. In innate immunity, autophagy is a mechanism to deliver cytosolic microbes to the lysosome, where they are killed by proteolytic enzymes. In adaptive immunity, autophagy is one of several mechanisms for generating microbial peptides in lysosomes for presentation to T cells.

Gain-of-function mutations in the gene that encodes STING result in systemic inflammatory disease with manifestations in skin and lung, driven by excess type I IFN production. This disorder is one example of a group of diseases characterized by excess type I IFN production, called interferonopathies, others of which are caused by genetic defects that result in increased amounts of nucleic acids in cells (e.g., mutations affecting endonucleases). Interferonopathies are part of a broader group of disorders called **autoinflammatory syndromes** that are characterized by spontaneous cytokine-driven inflammation without an overt inciting trigger. Autoinflammatory diseases are distinct from autoimmune diseases, which are disorders of adaptive immunity caused by antibodies and/or T cells reactive with self antigens. However, some autoinflammatory diseases, including some interferonopathies, are associated with autoimmune diseases, such as systemic lupus erythematous. This probably reflects the fact that innate immune responses are needed in addition to antigen to initiate T and B cell responses, and thus dysregulated production of innate cytokines that occurs in autoinflammatory syndromes may contribute to inappropriate activation of self-reactive lymphocytes and subsequent autoimmune disease.

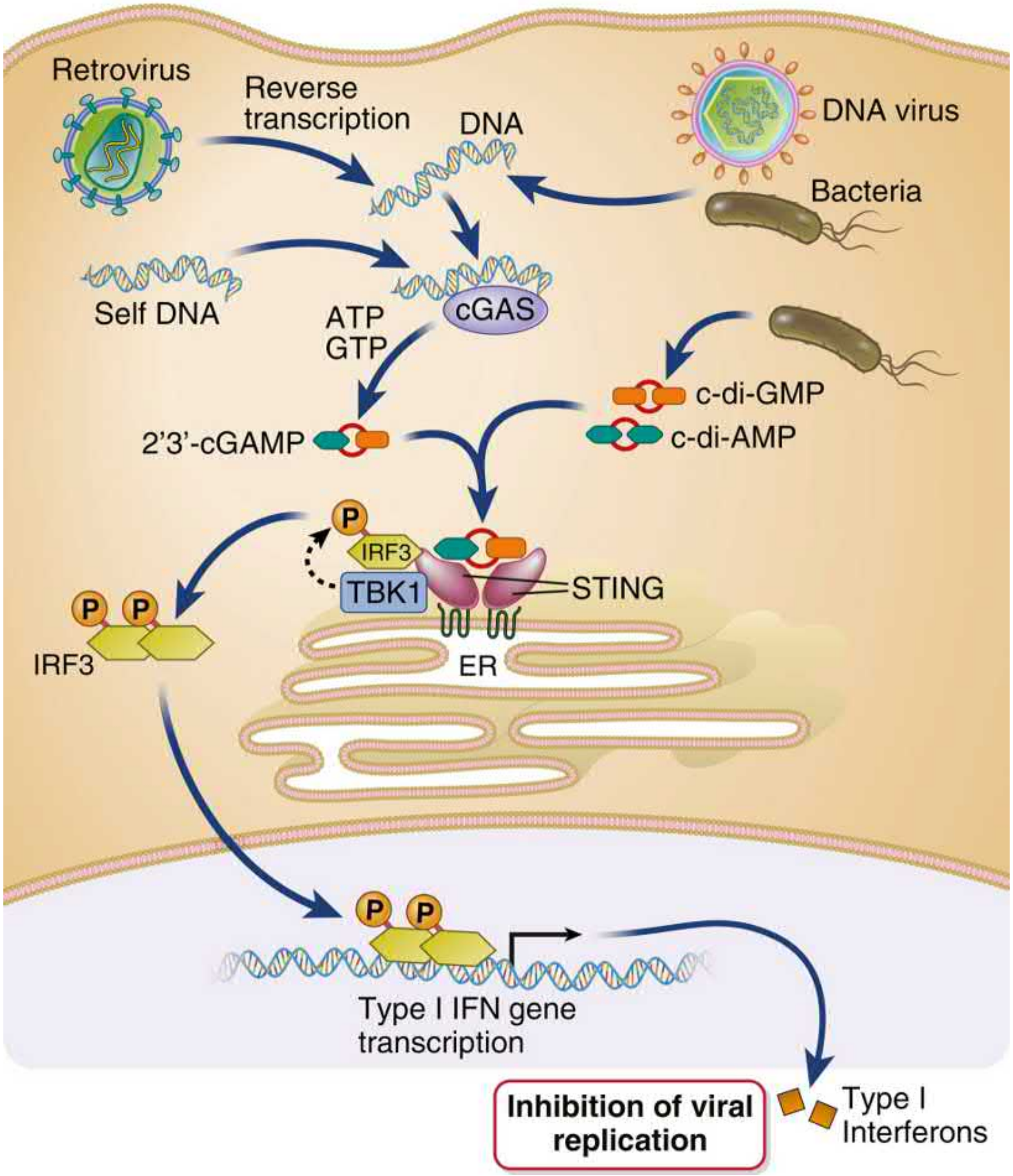

Retrovirus
Reverse transcription
DNA
DNA virus
Bacteria
Self DNA
ATP
GTP
cGAS
c-di-GMP
c-di-AMP
2'3'-cGAMP
P
IRF3
TBK1
STING
P
P
IRF3
ER
P
P
Type I IFN gene transcription
Inhibition of viral replication
Type I Interferons

FIGURE 4.5 The STING cytosolic DNA sensing pathway.Cytoplasmic microbial DNA and self DNA that accumulates in the cytosol activate the enzyme cGAS, which catalyzes the synthesis of cyclic GMP-AMP *(cGAMP)* from ATP and GTP. cGAMP binds to STING in the endoplasmic reticulum membrane, causing STING to translocate to the Golgi (not shown), and then STING recruits and activates the kinase TBK1,

which phosphorylates IRF3. Phospo-IRF3 moves to the nucleus, where it induces type I IFN gene expression. Self DNA may be produced as a result of genomic or mitochondrial damage or from turnover of DNA. The bacterial second messenger molecules cyclic di-GMP *(c-di-GMP)* and cyclic di-AMP *(c-di-AMP)* are directly sensed by STING. *ATP,* Adenosine triphosphate; *cGAS,* cyclic GMP-AMP synthase; *ER,* endoplasmic reticulum; *GTP,* guanosine triphosphate; *IFN,* interferon; *IRF3,* interferon response factor 3.

Some cytosolic DNA sensors may work through STING independent pathways.

- RNA polymerase 3 binds and transcribes AT-rich microbial dsDNA into an RNA-containing-triphosphate moiety, which then activates the RIG-I pathway leading to type I IFN expression, as described later.
- AIM2 (absent in melanoma-2) is another cytosolic sensor that binds cytosolic dsDNA and forms an inflammasome, which proteolytically generates the biologically active inflammatory cytokine IL-1β. Inflammasomes are also formed with other innate immune sensors and are described later.

RIG-Like Receptors

RIG-like receptors (RLRs) are cytosolic sensors of viral RNA that respond by inducing the production of the antiviral type I IFNs. This family of receptors is named after RIG. RLRs can recognize double-stranded RNA and RNA-DNA heteroduplexes, which are present in the genomes of RNA viruses and RNA transcripts of RNA and DNA viruses. The two best characterized RLRs are RIG-I and MDA5 (melanoma differentiation-associated gene 5). Both of these proteins contain two N-terminal caspase recruitment domains that interact with other signaling proteins, an RNA-helicase domain, and a C-terminal domain, the latter two being involved in RNA recognition. RIG-I and MDA5 recognize different sets of viral RNAs that are characteristic of distinct viruses and not typical of mammalian RNA. For example, MDA5 recognizes long dsRNA (1 to 6 kb), which is longer than dsRNA that may be formed transiently in normal cells, and RIG-I will only recognize RNA with a 5′ triphosphate moiety, which is not present in mammalian host cell cytosolic mRNA because of addition of a 7-methylguanosine cap. RIG-I can recognize uncapped short single-stranded RNA or dsRNA. MDA-5 can recognize dsRNAs with or without a 5′ cap but only if they are unmethylated at the 2′-O position on the ribose in the first nucleotide adjacent to the cap. Coronaviruses can evade RIG-I by utilizing enzymes that generate a 5′ cap on viral RNAs, and they evade MDA-5 mediated antiviral responses by expressing an enzyme that methylates the ribose in the first nucleotide of each viral RNA. RLRs are expressed in a wide variety of cell types, including bone marrow–derived leukocytes and various tissue cells. Therefore, these receptors enable the many cell types that are susceptible to infection by RNA viruses to mount innate immune responses to these viruses.

On binding viral dsRNA, the RLRs are recruited to the outer mitochondrial membrane by the MAVS (mitochondrial antiviral-signaling) protein. This leads to a self-perpetuating mechanism whereby MAVS polymerizes to form high-molecular-weight filamentous aggregates that induce other MAVS molecules to polymerize. The MAVS aggregates initiate signaling events that lead to phosphorylation and activation of IRF3 and IRF7, as well as NF-κB, and these transcription factors induce production of type I IFNs. MDA5 and RIG-I not only induce type I IFN production but also directly inhibit viral replication by inhibiting viral RNA-protein interactions.

Inflammasomes

Inflammasomes are multiprotein enzymatic complexes that form in the cytosol in response to infections or cell injury, which produce

proteolytically active caspase-1 and thereby generate biologically active forms of the inflammatory cytokines IL-1β and IL-18 (Fig. 4.6). IL-1β and IL-18 are two homologous cytokines that are produced as inactive precursors and must be proteolytically cleaved by the enzyme caspase-1 to become active cytokines that are released from the cell and promote inflammatory responses. Most inflammasomes (so-called canonical inflammasomes) are composed of oligomers of a sensor, caspase-1, and an adaptor that links the two, and these oligomeric complexes form only when the sensors detect changes in the cell indicating the presence of infection or damage. Thus, inflammation mediated by IL-1β and IL-18 occurs when there are PAMPs or DAMPs in the cytosol, indicating infection or cell injury.

Inflammasomes can form with several different sensor proteins. NLR family sensors found in different inflammasomes include NLRB, NLRC4, and at least six NLRP proteins (see Fig. 4.4). Sensors that are not in the NLR family but are used by other inflammasomes include members of the AIM2 family, including AIM2 and IFI16, which we discussed earlier as DNA sensors. These proteins contain a DNA sensing domain and a pyrin domain. Pyrin is another non-NLR sensor protein that contains an N-terminal pyrin domain and participates in the formation of an inflammasome. The gene encoding pyrin is mutated in familial Mediterranean fever, as discussed later.

The formation of the inflammasome is induced either when sensor proteins in the cytosol directly recognize microbial products or when sensors detect changes in the amount of endogenous molecules or ions in the cytosol that indirectly indicate the presence of infection or cell damage. In response to the PAMPs or indirect signals, the sensors become able to bind other proteins through homotypic interactions between shared structural domains, thereby forming the inflammasome complex. For example, after binding of a ligand, multiple identical NLRP3 proteins interact to form an oligomer, and each NLRP3 protein in the oligomer binds an adaptor protein called ASC (apoptosis-associated speck-like protein containing a CARD). The binding of

ASC to sensors such as NLRP proteins causes ASC to undergo a conformational alteration that triggers similar conformational changes of other ASC molecules in the cytosol by a self-propagating mechanism. This results in the formation of filaments of ASC that can cluster and recruit an inactive precursor of caspase-1 called pro–caspase-1. The recruitment and consequent clustering of pro–caspase-1 result in the proteolytic generation of active caspase-1. Caspases are proteases with cysteine residues in their active site that cleave substrate proteins at aspartate residues. Although several other caspases participate in a form of cell death called apoptosis (see Chapter 15), the main function of caspase-1 is to cleave the inactive cytoplasmic precursor forms of IL-1β and IL-18. Caspase-1 cleavage generates active forms of these cytokines, which then leave the cell and perform various proinflammatory functions. IL-1β lacks a signal peptide required for the secretion of most proteins from cells; thus, another mechanism is required for its release from the cytosol. Caspase-1 also cleaves a cytosolic protein called **gasdermin D,** generating an N-terminal fragment that polymerizes to form pores in the plasma membrane through which processed IL-1β may leave the cell. In some cell types, the gasdermin pores also contribute to a form of cell death called pyroptosis, described later. We will describe the action of IL-1β and IL-18 and the inflammatory response in detail later in this chapter. Suffice it to say here that the inflammation induced by IL-1 serves a protective function by recruiting phagocytes that eliminate the microbes and damaged cells that incited the formation of the inflammasome.

Inflammasome activation is induced by a wide variety of cytoplasmic stimuli that are often associated with infections and cell stress, including microbial products, environmentally or endogenously derived crystals, and reduction in cytosolic potassium ion (K^+) concentrations (see Fig. 4.6) **.** NLRC4 recognizes cytosolic flagellin and components of the type III secretion system of bacteria. NLRP1 recognizes the anthrax lethal toxin. NLRP3 senses many DAMPS and PAMPS, including uric acid crystals, aluminum hydroxide crystals used in vaccine adjuvants, adenosine triphosphate (ATP) released from mitochondria, silica, bacterial products, bacterial toxins produced by streptococci and staphylococci, bacterial DNA-RNA hybrids, and the influenza virus. Pyrin responds to numerous bacterial toxins that all mediate post-translational modification of endogenous Rho family GTPases.

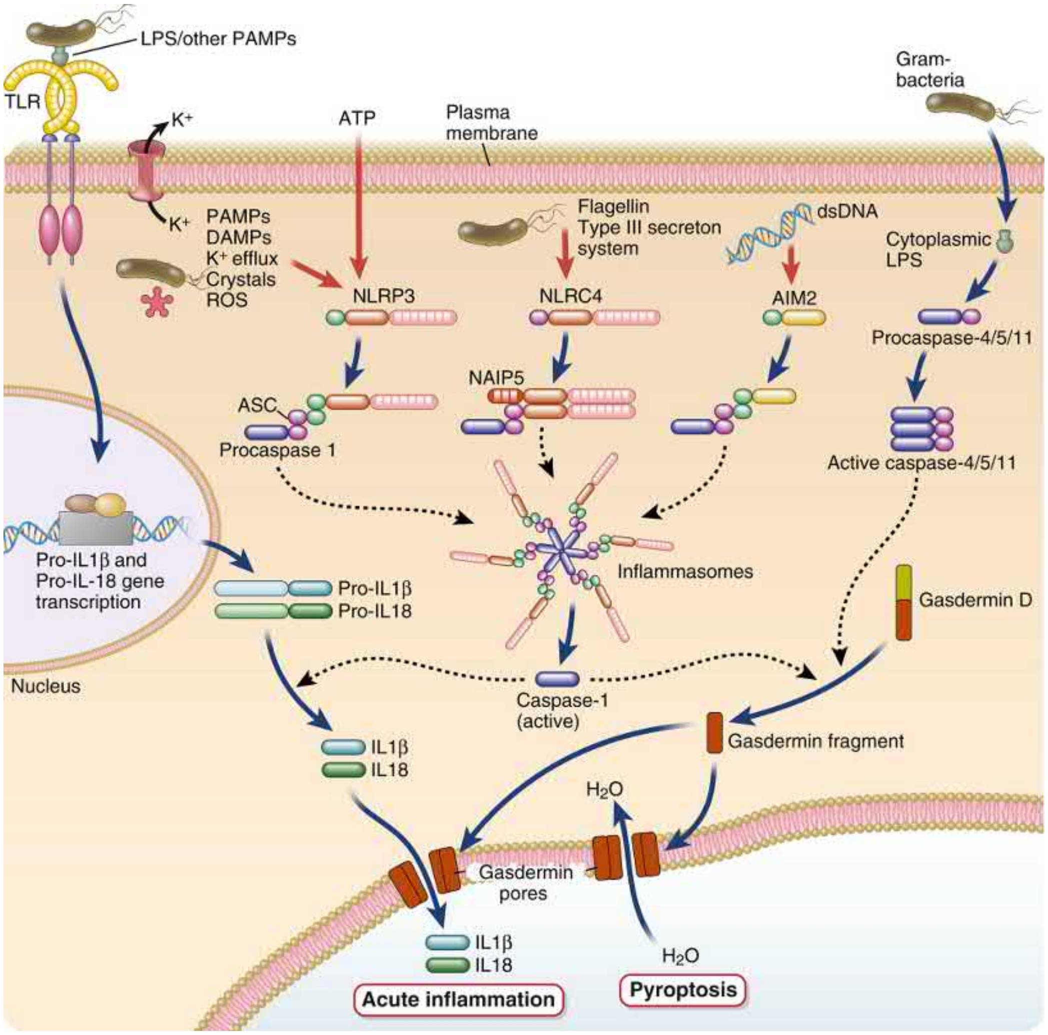

LPS/other PAMPs
TLR
K+
K+
ATP
Plasma membrane
Gram-bacteria
PAMPs
DAMPs
K+ efflux
Crystals
ROS
Flagellin
Type III secreton system
dsDNA
Cytoplasmic LPS
NLRP3
NLRC4
AIM2
Procaspase-4/5/11
NAIP5
ASC
Procaspase 1
Active caspase-4/5/11
Inflammasomes
Pro-IL1β and Pro-IL-18 gene transcription
Pro-IL1β
Pro-IL18
Gasdermin D
Nucleus
Caspase-1 (active)
Gasdermin fragment
IL1β
IL18
H2O
Gasdermin pores
IL1β
IL18
H2O
Acute inflammation
Pyroptosis

FIGURE 4.6 Inflammasomes.Activation of three different canonical inflammasomes using NLRP3, NLRC4, or AIM2 as sensors, and the noncanonical inflammasomes composed of caspases 4, 5, or 11 are shown. Some of the ligands or cellular conditions that induce the assembly each of these inflammasomes are indicated. The canonical inflammasomes assemble as multimeric complexes including the NLR sensors, adaptor proteins such as ASC or NIAP5, and procaspase 1, leading to proteolytic generation of active caspase 1, which processes pro–IL-1β and pro–IL-18 to active IL-1β and IL-18. Inflammasome-activated caspase-1 can also

proteolytically cleave the cytosolic protein gasdermin D, generating an N-terminal fragment that polymerizes in the plasma membrane, forming a pore that lets IL-1β out of and water and ions into the cell, leading to cell death by osmotic lysis. This pathway of cell death is called pyroptosis, because it is accompanied by inflammation due to IL-1 released from the dying cells. Cytoplasmic LPS induces the assembly of procaspase-4, -5, or -11 molecules to form nonconical inflammasomes that are multimers of active caspase 4, 5, or 11, which can also cleave gasdermin D, leading to gasdermin-N pore formation and pyrotosis. *AIM2,* Absent in melanoma-2; *ASC,* apoptosis-associated speck-like protein containing a CARD; *ATP,* adenosine triphosphate; *DAMPs,* damage-associated molecular patterns; *IL-1*, interleukin-1; *LPS,* lipopolysaccharide; *NLR,* NOD-like receptor; *PAMPs,* pathogen-associated molecular patterns; *ROS,* reactive oxygen species; *TLR,* Toll-like receptor.

The mechanism by which such varied molecules activate the same NLR sensors is unclear. The structural diversity of the agents that activate these sensors suggests that they do not all directly bind to NLR proteins but may act by inducing a shared set of changes in endogenous cytoplasmic conditions that activate the NLRs. Reduced cytoplasmic potassium ion concentration may be one such common mechanism, because reductions in cellular K^+ induced by some bacterial pore-forming toxins can activate inflammasomes, and many of the other known inflammasome activators, such as extracellular ATP, cause increased K^+ efflux from cells. Another common mechanism implicated in inflammasome activation is the generation of reactive oxygen species (ROS), which are toxic free radicals of oxygen that are often produced during cell injury. Inflammasome-activating crystals may work by damaging lysosomal membranes, thereby releasing ROS into the cytosol, where they are detected by the sensors that stimulate inflammasome formation.

Inflammasome activation of caspases also causes an inflammatory form of programmed cell death of macrophages and DCs (but not of neutrophils and most other cell types) called pyroptosis (see Fig. 4.6) **.** This form of cell death is the result of plasma membrane pores formed by caspase-generated fragments of gasdermin D, mentioned earlier as a pathways for IL-1β release from cells. These pores contribute to osmotic death of macrophages and DCs, characterized by influx of fluid, swelling of cells, loss of plasma membrane integrity, and release of inflammatory mediators, including IL-1β, IL-18, TNF, IL-6, and IL-8. Pyroptosis also results in the death of certain microbes that gain access to the cytosol. Pyroptosis is induced by canonical inflammasomes using the sensors NLRC4, NLRP1, AIM2, pyrin, and NLRP3, all leading to activation of caspase-1, which cleaves gasdermin D, removing the inhibitory C-terminal domain and generating the pore-forming N-terminal domain. Pyroptosis also may be induced by activation of a noncanonical inflammasome pathway that uses a different caspase (caspase-11 in rodents, caspase-4 or caspase-5 in humans). Bacterial LPS in the cytosol can directly bind to caspase-11, which leads to proteolytic activation of gasdermin D and pore formation causing pyroptosis and also indirectly leads to NLRP3 inflammasome activation and generation of active IL-1β. The amplification of inflammation provided by pyroptosis enhances bacterial clearance but may also contribute to septic shock, a severe systemic reaction to inflammatory cytokines. The absence of caspase-11 in genetically engineered mice protects them from LPS-induced septic shock.

The discovery that some crystalline substances are potent inflammasome activators has changed our understanding of certain inflammatory diseases. **Gout** is a painful inflammatory condition of the joints that has long been known to be associated with deposition of monosodium urate crystals in joints. Experimental evidence suggests that when these crystals are phagocytosed, they damage the lysosomal membranes of the cells, and this leads to activation of inflammasomes and subsequent inflammation. Based on these findings, IL-1 antagonists have been used to effectively treat cases of severe gout that are resistant to conventional antiinflammatory drugs. Similarly, pseudogout is caused by deposition of calcium pyrophosphate crystals and inflammasome activation. Occupational inhalation of silica and asbestos can cause chronic inflammatory and fibrotic disease of the lung, and there is also interest in the potential of blocking the inflammasome or IL-1 to treat these diseases.

Dysregulated activation of inflammasomes, most often due to autosomal gain-of-function mutations in one or another of their component proteins, leads to inappropriately triggered and excess IL-1 production. The result is recurrent attacks of fever and localized inflammation, most commonly in the skin, joints, and abdominal cavity. These disorders are called inflammasomopathies or IL-1β–activation syndromes, and are, like type 1 interferonopathies discussed earlier, autoinflammatory syndromes. The longest studied of the inflammasome-related autoinflammatory syndromes is familial Mediterranean fever, caused by mutation of the *MEFV* gene, which encodes pyrin. Autoinflammatory diseases caused by mutations in NLRP3 (also known as cryopyrin) are called cryopyrin-associated periodic syndromes (CAPS). Patients with CAPS can be successfully treated with IL-1 antagonists, as predicted from the pathogenesis of the syndromes.

A great deal of interest in inflammasomes has recently been generated by findings that they may be activated by excessive amounts of endogenous substances deposited in tissues in the setting of various diseases. These substances include cholesterol crystals within macrophages in atherosclerosis, free fatty acids and lipids in adipose tissue in obesity-associated metabolic syndrome and type 2 diabetes, and β-amyloid in Alzheimer's disease. In all of these situations, activation of inflammasomes leads to production of IL-1 and inflammation, which may contribute to the pathogenesis of the diseases. Such findings have spurred clinical trials to alleviate some of these diseases using IL-1 antagonists.

Other Cell-Associated Pattern Recognition Receptors

Several other types of plasma membrane and cytoplasmic receptors transmit activating signals similar to TLRs that promote inflammatory responses and enhance killing of microbes or mainly participate in the uptake of microbes into phagocytes (see Table 4.3).

C-Type Lectins: Receptors for Microbial Carbohydrates

Cellular receptors that recognize carbohydrates on the surface of microbes facilitate the phagocytosis of the microbes and the secretion of cytokines that promote inflammation and subsequent adaptive immune responses (Table 4.4) **.** These receptors belong to the C-type lectin family, so called because they bind carbohydrates (hence lectins) in a Ca^{++}-dependent manner (hence C-type). The C-type lectins are also called C-type lectin-like receptors or CLRs to parallel the nomenclature of TLRs and other pattern recognition receptors. These receptors are integral membrane proteins found on the surfaces of macrophages, DCs, and some tissue cells. Other lectins are soluble proteins in the blood and extracellular fluids (discussed later). All of these molecules contain a conserved carbohydrate recognition domain. There are several types of plasma membrane C-type lectins with specificities for different carbohydrates, including mannose, glucose, N-acetylglucosamine, and β-glucans. In general, these cell surface lectins recognize carbohydrate structures found on the cell walls of microorganisms but not mammalian cells. Some of these C-type lectin receptors function in the phagocytosis of microbes, and others have signaling functions that induce protective responses of host cells to microbes.

TABLE 4.4

	Mannose Receptor (CD206)	Dectin-1 (CD369)	Dectin-2 and Mincle	DC-Sign (CD209)	Langerin (CD207)
Ligand	Terminal mannose and fucose on microbial cell surfaces	β-Glucan on fungal cell surfaces	High mannose on fungal hyphae and bacteria	Terminal mannose and fucose on microbial cell surfaces	Terminal mannose on microbial cell surfaces
Signaling	Uncertain	ITAM/SYK/CARD9 pathway of NF-κB activation	ITAM/SYK/CARD9 pathway of NF-κB activation	Uncertain	Uncertain
Cellular expression	Macrophages	Dendritic cells	Dendritic cells	Dendritic cells, macrophages and sinusoidal endothelial cells	Dendritic cells in epithelial barriers
Function	Phagocytosis; antifungal immunity	Inflammation and antigen presentation; Th17 differentiation; antifungal immunity	Inflammation and antigen presentation; Th17 differentiation; antifungal and mycobacterial immunity	Adhesion; hepatitis C virus and HIV-1 infection	Phagocytosis, antigen presentation

DC-SIGN, Dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin; *ITAM*, immunoreceptor tyrosine-based activation motif; *mincle*, macrophage-inducible Ca^{++}-dependent lectin.

- The **mannose receptor** (CD206) is expressed on macrophages, DCs, and lymphatic endothelial cells and is involved in phagocytosis of various types of microbes. This receptor recognizes certain terminal sugars on microbial surface carbohydrates, including D-mannose, L-fucose, and *N*-acetyl-D-glucosamine. These terminal sugars are often present on the surface of microorganisms, whereas eukaryotic cell surface carbohydrates are most often terminated by galactose and sialic acid. Thus, the terminal sugars on microbes can be considered PAMPs. Signaling functions of the mannose receptor are not well described, and the short cytoplasmic tail of the receptor contains no known signaling motifs.
- **Dectins** (DC-associated C-type lectins) include several different lectins encoded within a gene cluster on human chromosome 12, which also includes genes encoding NK cell receptors (discussed later). Dectins are expressed on DCs and macrophages, and play important roles in antifungal immunity, as well as in responses to certain bacteria. Dectin-1 (CD369) binds β-glucan, which is a major

cell wall component of many fungal species and is required for immunity to various pathogenic fungi, including *Candida*, *Aspergillus*, and *Pneumocystis*. Dectin-2 and Mincle are two dectins that recognize high-mannose oligosaccharides on the hyphal form of some fungi and on bacteria. In response to binding of their ligands, these dectins induce signaling events in DCs and macrophages that activate a variety of immune functions. The cytoplasmic tail of Dectin-1 has an immunoreceptor tyrosine-based activation motif (ITAM), which engages tyrosine kinases, which transduce signals leading to gene transcription. Dectin-2 and Mincle rely on an associated ITAM-bearing signaling partner protein called FcRγ. ITAMs are used by many different cell-activating receptors in the immune system, and their mechanism of signaling will be discussed in Chapter 7. In response to ligand binding to Dectin-1, Dectin-2, or Mincle, DCs produce cytokines and other proteins that promote inflammation and enhance adaptive immune responses. Some of the induced cytokines promote the development of a type of effector T cell called Th17, which is particularly effective in defense against fungal and some bacterial infections (see Chapter 10).

- **Langerin** (CD207) and **DC-SIGN** (CD209) are two other lectins expressed on DCs, both of which bind mannose and have roles in immune responses to various microbes. Langerin is expressed by epidermal Langerhans cells and other subsets of DCs in skin and other epithelial barriers. DC-SIGN is expressed on the majority of DCs as well as on macrophages and sinusoidal endothelial cells. DC-SIGN binds to envelope glycoproteins of the hepatitis C virus and HIV-1 and may play a pathogenic role in disseminating infection by these viruses.

Scavenger Receptors

Scavenger receptors comprise a structurally and functionally diverse collection of cell surface proteins that were originally grouped on the basis of the common characteristic of mediating the uptake of oxidized lipoproteins into cells. Some of these scavenger receptors, including scavenger receptor A (SR-A) and CD36, are expressed on macrophages and mediate the phagocytosis of microorganisms. In addition, CD36 functions as a coreceptor in TLR2/6 recognition and response to bacterially derived lipoteichoic acid and diacylated lipopeptides. A wide range of molecular structures bind to each scavenger receptor, including LPS, lipoteichoic acid, nucleic acids, β-glucan, and proteins. The significance of scavenger receptors in innate immunity is highlighted by increased susceptibility to infection in gene knockout mice lacking these receptors and by the observation that several microbial pathogens express virulence factors that block scavenger receptor–mediated recognition and phagocytosis.

Formyl-Peptide Receptors

The **formyl peptide receptor-1** (FPR1), expressed on leukocytes, recognizes bacterial peptides containing *N*-formylmethionyl residues and stimulates directed movement of the cells. Because all bacterial proteins and few mammalian proteins (only those synthesized within mitochondria) are initiated by *N*-formylmethionine, FPR1 enables phagocytes to detect and respond preferentially to bacterial proteins. The bacterial peptide ligands that bind this receptor are some of the most potent known chemoattractants for leukocytes. Chemoattractants include several types of diffusible molecules, often produced at sites of infection, that bind to specific receptors on cells and direct their movement toward the source of the chemoattractant. Other chemoattractants, such as the chemokines discussed in Chapter 3, are made by host cells. FPR1 and all other chemoattractant receptors belong to the seven-transmembrane, guanosine triphosphate (GTP)–binding (G) protein–coupled receptor (GPCR) superfamily. These receptors initiate intracellular responses through associated trimeric G proteins (see Chapter 7). The G proteins stimulate many types of cellular responses, including cytoskeletal changes that are responsible for the increased cell motility.

Cellular Components of the Innate Immune System

The three main functions of the cells of the innate immune system are to serve as barriers against infections, act as sentinels to detect microbes and damaged cells in tissues, and perform effector functions that eliminate microorganisms. Innate immune cells express the various pattern recognition receptors we have just discussed, and, after recognizing PAMPs and DAMPs, they respond by producing inflammatory cytokines and antiviral proteins and kill microbes or infected host cells. In addition, some of the cells of innate immunity are critical for stimulating subsequent adaptive immune responses.

Epithelial Barriers

Intact epithelial surfaces form physical barriers between microbes in the external environment and host tissue (Fig. 4.7) **.** The main interfaces between the environment and the mammalian host are the skin and the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts. These interfaces are lined by continuous layers of specialized epithelial cells that serve many physiologic functions, including preventing the entry of microbes. Loss of the integrity of these epithelial layers by trauma or other reasons predisposes an individual to infections. We will summarize the main features of innate defense by epithelial barriers here and discuss epithelial barrier immunity in greater detail in Chapter 14.

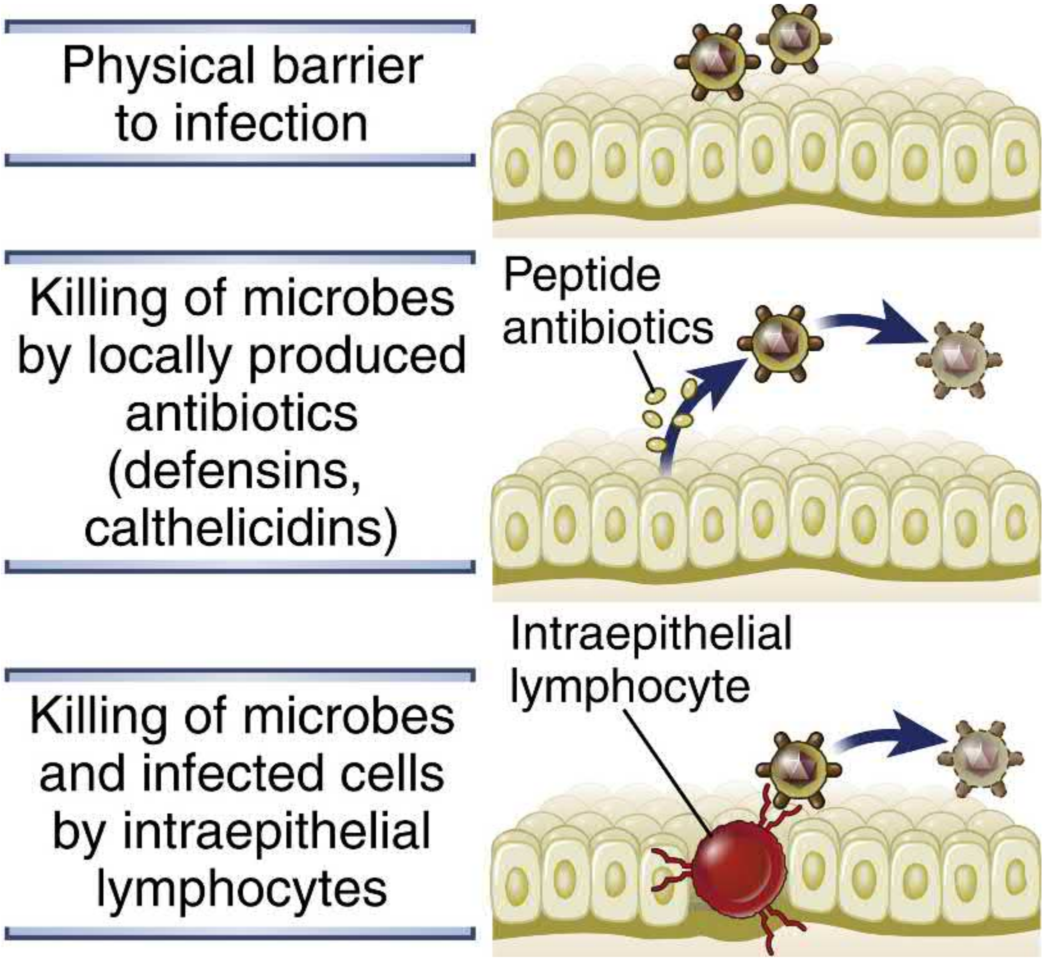

Physical barrier to infection
Killing of microbes by locally produced antibiotics (defensins, calthelicidins)
Peptide antibiotics
Killing of microbes and infected cells by intraepithelial lymphocytes
Intraepithelial lymphocyte

FIGURE 4.7 Epithelial barriers.Epithelia at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor intraepithelial lymphocytes that are believed to kill microbes and infected cells.

The protective function of barrier epithelia is in large part physical. The epithelial cells form tight junctions with one another, blocking passage of microbes between the cells. In the skin, the outer layer of keratin, which accumulates as keratinocytes on the surface die, serves to block microbial penetration into deeper layers of the epidermis, and cutaneous infections generally occur when there is a barrier breach. Mucus, a viscous secretion containing glycoproteins called mucins, is produced by respiratory, gastrointestinal, and urogenital epithelial cells and physically impairs microbial invasion. The function of these barriers is enhanced by ciliary action in the bronchial tree and peristalsis in the gut, which facilitate elimination of microbes. Although these physical properties alone are very important in host defense, other epithelial defense mechanisms have evolved to complement the mechanical barrier.

Epithelial cells, as well as some leukocytes, produce peptides that have antimicrobial properties. Two structurally distinct families of antimicrobial peptides are the defensins and the cathelicidins.

- **Defensins** are small peptides, 29 to 34 amino acids long, that contain both cationic and hydrophobic regions and three intrachain disulfide bonds. Two families of human defensins, named α and β, are distinguished by the location of these bonds. Defensins are produced by epithelial cells of mucosal surfaces and by granule-containing leukocytes, including neutrophils, NK cells, and cytotoxic T lymphocytes (CTLs). The set of defensin molecules produced differs among different cell types. Paneth cells within the crypts of the small bowel are a major producer of α-defensins. Paneth cell defensins are sometimes called crypticidins; their function is to limit the amount of luminal microbes near the epithelial barrier. Defensins are also produced in the colon, in respiratory mucosal cells, and in the skin. Some defensins are constitutively produced by some cell types, but their secretion may be enhanced by cytokines and microbial products. In other cells, defensins are produced only in response to cytokines and microbial products. The protective actions of the defensins include both direct toxicity to microbes, including bacteria, fungi, and enveloped viruses, and the activation of cells involved in the inflammatory response to microbes. Defensins kill microbes by a variety of mechanisms, many of which depend on their ability to insert into and disrupt functions of microbial membranes.

- **Cathelicidin,** produced by neutrophils and barrier epithelial cells in the skin, gastrointestinal tract, and respiratory tract, is synthesized as an 18-kD precursor protein that is secreted and then proteolytically cleaved into two peptides, each with protective functions. Both precursor synthesis and proteolytic cleavage may be stimulated by inflammatory cytokines and microbial products. The active cathelicidins protect against infections by multiple mechanisms, including direct toxicity to a broad range of microorganisms and the activation of various responses in leukocytes and other cell types that promote eradication of microbes. The C-terminal fragment, called LL-37, can bind and neutralize LPS, the toxic component of the outer wall of gram-negative bacteria that is recognized by TLR4.

Barrier epithelia contain certain types of lymphocytes, including intraepithelial T lymphocytes, which recognize and respond to commonly encountered microbes. Intraepithelial T lymphocytes are present in the epidermis of the skin and in mucosal epithelia. Various subsets of intraepithelial lymphocytes are present in different proportions, depending on species and tissue location. These subsets are distinguished mainly by the type of antigen receptors they express. Some intraepithelial T lymphocytes express the conventional $\alpha\beta$ form of the T cell receptor (TCR), which is present on most T cells in lymphoid tissues and in the circulation. Other T cells in epithelia express a form of antigen receptor called the $\gamma\delta$ TCR that may recognize peptide and nonpeptide antigens. A characteristic of intraepithelial T cells is the limited diversity of their antigen receptors, compared with most T cells in the adaptive immune system. These intraepithelial T lymphocytes are thought to recognize a small number of commonly encountered microbial structures, a typical feature of innate pattern recognition receptors we have described. It is also possible that these lymphocytes are activated not by antigen recognition but by cytokines and other molecules produced by epithelial cells in response to stress.

Intraepithelial lymphocytes may function in host defense by secreting cytokines, activating phagocytes, and killing infected cells.

Phagocytes

Cells that have specialized phagocytic functions, primarily macrophages and neutrophils, eliminate microbes that breach epithelial barriers. We introduced these cell types in Chapter 2, and we will discuss many other details of their functions in the context of the inflammatory response later in this chapter. The essential role that phagocytes play in innate immune defense against microbes is demonstrated by the high rate of bacterial and fungal infections in patients with low blood neutrophil counts caused by bone marrow cancers or chemotherapy and irradiation for cancer (which destroys immature cells in the bone marrow) and in patients with inherited deficiencies in the functions of neutrophils and macrophages. Neutrophils and monocytes are circulating phagocytes that are recruited into tissues in response to signals generated by innate sentinel cells. The monocytes rapidly differentiate into macrophages after leaving the blood. Tissue-resident macrophages are always present in most tissues under normal conditions and also serve as sentinel cells.

Dendritic Cells

Dendritic cells rapidly and efficiently detect invading microbes because of their location in tissues and their expression of numerous pattern recognition receptors for PAMPs and DAMPs. DCs express many different types of TLRs and cytoplasmic pattern recognition receptors, making them versatile sensors of PAMPs and DAMPs. In response to invading microbes, they secrete inflammatory cytokines that promote recruitment of additional leukocytes from the blood. The plasmacytoid subset of DCs is a major source of the antiviral cytokines type I IFNs, produced in response to viral infections. We will discuss the antiviral actions of type I IFNs in more detail later in the chapter.

The ability of DCs to promote T lymphocyte responses after innate immune activation also makes them an important bridge between innate and adaptive immunity. The reactions of DCs to microbes in the early innate response enhance the ability of the DCs to activate T cells in the subsequent adaptive immune response. DCs capture antigens and display them to T cells (see Chapter 6). DCs activated by microbial PAMPs also express membrane molecules called costimulators that promote T cell responses. In addition, depending on the nature of the microbe that induces the innate response, a DC will direct naive T cell differentiation into distinct types of effector cells, such as IFN-γ–producing Th1 cells or IL-17–producing Th17 cells. These features of DCs will be discussed in Chapters 9 and 10. Because of their tissue residence and ability to rapidly detect infections in the tissues, DCs are the prototypic sentinels of the immune system.

Cytokine-Producing Innate Lymphoid Cells

Innate lymphoid cells (ILCs), which were introduced in Chapter 2, are bone marrow–derived cells with lymphocyte morphology that were discovered as cells that produced cytokines similar to those made by helper T cells but lacked TCRs. There are different subsets of ILCs that arise from the same common lymphoid precursor that gives rise to B and T cells, but the precise steps in ILC development are not fully understood, especially in humans. It is clear that during their development, there are branch points giving rise to three different "helper" subsets of ILCs, which function mainly by secreting different types of cytokines, similar to $CD4^+$ helper T cell subsets, and a separate branch giving rise to NK cells, which function as cytotoxic effectors in addition to secreting the cytokine IFN-γ, similar to $CD8^+$ CTLs. We will describe cytokine-producing ILC subsets in this section and NK cells in the following section.

Three subsets of ILCs, called ILC1, ILC2, and ILC3, produce different cytokines and express different transcription factors, analogous to the Th1, Th2, and Th17 subsets of CD4 $^+$ T

lymphocytes (Fig. 4.8) **.** ILC1s produce IFN-γ and express the transcription factor T-BET, like Th1 cells. ILC2s produce mainly IL-5 and IL-13. and express the transcription factor GATA3, like Th2 cells. ILC3s produce IL-22 and/or IL-17 and express the transcription factor RORγt, like Th17 cells. Because ILCs do not express T cell receptors, they must be activated by different mechanisms than helper T cells to produce these cytokines. The best-defined stimuli for ILC cytokine production are other cytokines, sometimes called alarmins, which are released in the context of innate responses to infections and tissue damage; each ILC subset is activated by different cytokines (see Fig. 4.8).

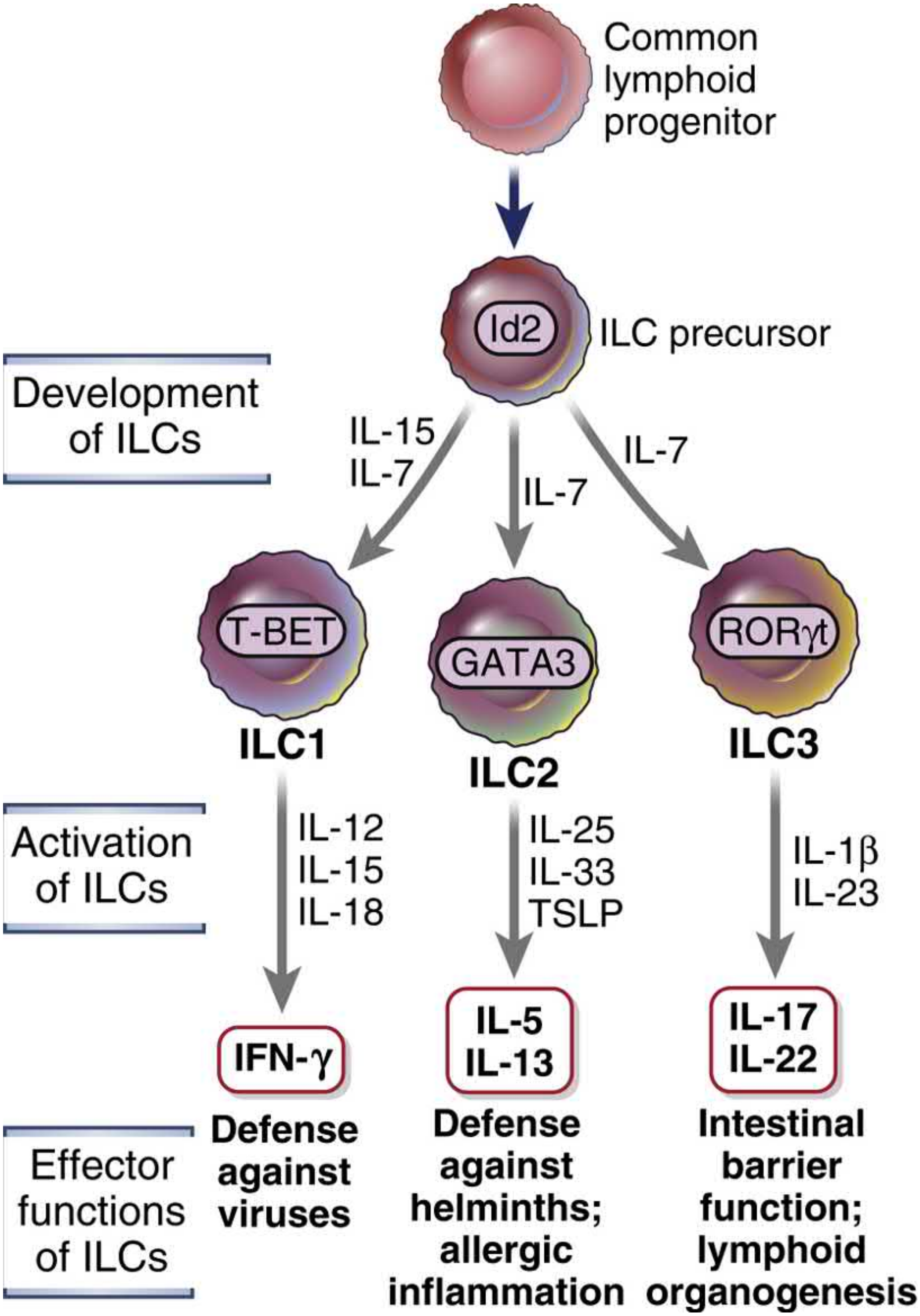

Common lymphoid progenitor
Id2
ILC precursor
Development of ILCs
IL-15
IL-7
IL-7
IL-7
T-BET
GATA3
RORγt
ILC1
ILC2
ILC3
Activation of ILCs
IL-12
IL-15
IL-18
IL-25
IL-33
TSLP
IL-1β
IL-23
IFN-γ
IL-5
IL-13
IL-17
IL-22
Effector functions of ILCs
Defense against viruses
Defense against helminths; allergic inflammation
Intestinal barrier function; lymphoid organogenesis

FIGURE 4.8 Cytokine producing innate lymphoid cell subsets. The three major subsets of cytokine producing innate lymphoid cells *(ILCs)* develop from the common lymphoid progenitor that also

gives rise to B and T lymphocytes and natural killer (NK) cells (not shown). A common ILC precursor identified by the Id2 transcription factor differentiates into three major subsets of cytokine-producing ILCs. Each differentiated subset is distinguished by expression of distinct transcription factors and by cytokines produced when activated, as indicated. The cytokines that drive differentiation into ILC1, 2, or 3 subsets, as well as the cytokines that activate ILCs to produce their own subset-specific cytokines, are shown. The major known functions of the ILCs are also indicated. The cytokines indicated in bold are discussed in Chapter 10, in the context of T cell responses. The functions of the other cytokines mentioned in the figure are summarized later in this chapter (see Table 4.5), and all these cytokines are listed in Appendix I. *IFN,* Interferon; *IL,* interleukin; *TSLP,* thymic stromal lymphopoietin.

ILC subsets may participate in host defense against distinct pathogens and also may be involved in inflammatory disorders. ILC1s are likely important for defense against intracellular microbes. ILC2s are important for defense against helminthic parasites, and they also may contribute to allergic diseases. ILC3s are found at mucosal sites and participate in defense against extracellular fungi and bacteria, as well as in maintaining the integrity of epithelial barriers. Lymphoid tissue–inducer (LTi) cells are a subtype of ILC3s, which, in addition to secreting IL-17 and IL-22, also express the membrane molecule lymphotoxin and secrete TNF, both of which are required for the normal development of lymphoid organs (see Chapter 2).

The feature of ILCs that makes them potentially important for early host defense is that they are always resident in epithelial barrier tissues, poised to react against microbes that breach those barriers. In contrast, T cells circulate through secondary lymphoid organs and migrate into tissues only after they are activated and differentiate into effector cells, a process that may take several days after encounter with a microbe. Because corresponding subsets of ILCs and helper T cells produce similar cytokines, they may work in a temporally coordinated way, with ILCs being the early innate participants, activated by alarmins in infected tissues, and the helper T cells appearing later as part of adaptive immunity. The actions of ILC1s, NK cells, and Th1 cells are sometimes grouped under type I immunity (along with activated $CD8^+$ T cells, discussed in later chapters), ILC2s and Th2 cells are type II immunity, and ILC3s and Th17 cells comprise type III immunity. In general, although ILCs may be active early, effector T cells rapidly become the dominant participants because they are more numerous and make much larger amounts of cytokines. The contribution of ILCs to host defense has been difficult to establish because it has not been possible to selectively eliminate these cells or their cytokines in experimental animals without having an impact on the analogous T lymphocytes as well. The role of ILCs in host defense and inflammatory diseases in humans is also uncertain because these cells are numerically rare in blood and therefore difficult to study.

Natural Killer Cells

NK cells are cytotoxic cells that play important roles in innate immune responses, mainly against viruses and intracellular bacteria. The natural killer designation derives from the fact that their major function is killing infected cells, similar to the adaptive immune system's killer cells, the CTLs, but unlike naive $CD8^+$ T cells, NK cells are functionally competent to kill other cells when they are present in the blood or tissues without further differentiation (hence natural). NK cells also secrete IFN-γ and thus resemble ILC1s. They are also developmentally related to ILC1s but are not considered identical because they are defined based on cytotoxic activity, not cytokine production. Unlike ILCs, which are found in peripheral tissues but are rare in the blood and lymphoid organs, NK cells constitute 5% to 20% of lymphocytes in the blood and spleen. They are rare in other lymphoid organs and in most nonlymphoid tissues but are numerous in the liver and placenta. NK cells in the blood appear as large lymphocytes with numerous cytoplasmic granules. NK cells do not express diverse, clonally distributed antigen receptors typical of B and T cells. Rather, they use germline DNA–encoded receptors (discussed later) to distinguish pathogen-infected cells from healthy cells. They can be identified in the blood by expression of CD56 and the absence of the T cell marker CD3. Most human blood NK cells also express CD16, an IgG Fc receptor that is involved in recognition of antibody-coated cells.

Functions of Natural Killer Cells

The effector functions of NK cells are to kill infected cells and to produce IFN-γ, which activates macrophages to destroy phagocytosed microbes (Fig. 4.9) **.** The mechanism of NK cell–mediated cytotoxicity is essentially the same as that of CD8$^+$ CTLs, which we will describe in detail in Chapter 11. NK cells, like CTLs, have granules that contain proteins that mediate killing of target cells. When NK cells are activated, granule exocytosis releases these proteins adjacent to the target cells. One NK cell granule protein, called **perforin,** facilitates the entry of other granule proteins, called **granzymes,** into the cytosol of target cells. The granzymes are proteolytic enzymes that initiate a sequence of signaling events that cause death of the target cells by apoptosis. By killing cells infected by viruses and intracellular bacteria, NK cells eliminate reservoirs of infection. Early in the course of a viral infection, NK cells are expanded and activated by recognition of activating ligands on the infected cells and by the cytokines IL-12 and IL-15, and they kill infected cells before antigen-specific CTLs can become fully active, which usually takes 5 to 7 days. NK cells also may be important later in the course of viral infection by killing infected cells that have escaped CTL-mediated immune attack by reducing expression of class I major histocompatibility complex (MHC) molecules. Some tumors, especially those of hematopoietic origin, are targets of NK cells because the tumor cells upregulate ligands for activating NK receptors and do not express normal levels or types of class I MHC molecules, which inhibit NK cell activation, discussed later.

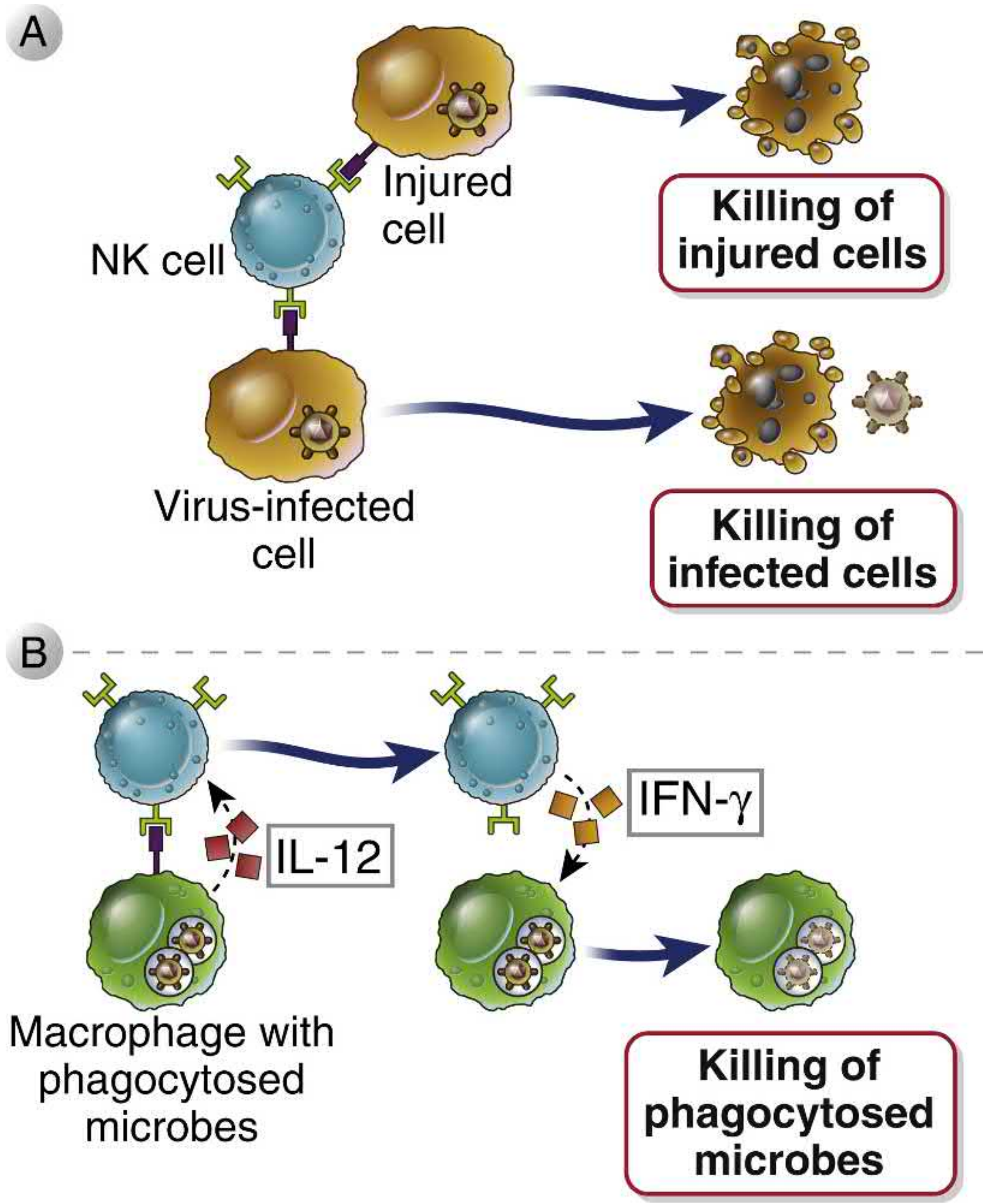

A
Injured cell
NK cell
Killing of injured cells
Virus-infected cell
Killing of infected cells
B
IL-12
IFN-γ
Macrophage with phagocytosed microbes
Killing of phagocytosed microbes

FIGURE 4.9 Functions of natural killer cells. **A,** Natural killer *(NK)* cells recognize ligands on infected cells or cells undergoing other types of stress and kill the host cells. In this way, NK cells eliminate reservoirs of infection as well as dysfunctional cells. **B,** NK cells respond to interleukin-12 (IL-12) produced by macrophages and secrete interferon- γ *(IFN-γ),* which activates the macrophages to kill phagocytosed microbes.

NK cell–derived IFN-γ increases the capacity of macrophages to kill phagocytosed bacteria, similar to IFN-γ produced by T cells (see Chapter 10). This IFN-γ–dependent NK cell–macrophage interaction can control an infection with intracellular bacteria for several days or weeks and thus allow time for T cell–mediated immunity to develop and eradicate the infection. IFN-γ produced by NK cells in lymph nodes can also direct the differentiation of naive T cells into Th1 cells (see Chapter 10). Some human NK cells do not express CD16, nor are they cytotoxic, but they do produce abundant IFN-γ. Predictably, deficiency of NK cells, seen in rare individuals, leads to increased susceptibility to infection by some viruses and intracellular bacteria.

Activating and Inhibitory Receptors of Natural Killer Cells

NK cells distinguish infected and stressed cells from healthy cells, and NK cell function is regulated by a balance between signals that are generated by activating and inhibitory receptors. In general, the activating receptors recognize ligands on infected and injured cells, and the inhibitory receptors recognize ligands on healthy normal cells (Fig. 4.10). When an NK cell interacts with another cell, the outcome is determined by the integration of signals generated from the array of inhibitory and activating receptors that are expressed by the NK cell and that interact with ligands on the other cell. Engagement of activating receptors stimulates the killing activity of the NK cells, resulting in destruction of stressed or infected cells. In contrast, engagement of inhibitory receptors shuts off NK cell activity and prevents destruction of healthy cells. Because of the stochastic nature of their expression, there is significant diversity in the array of activating and inhibitory receptors that different NK cells express in any one individual. The result of this is that individual NK cells, even in the same person, may respond to cells infected with different types of microbes.

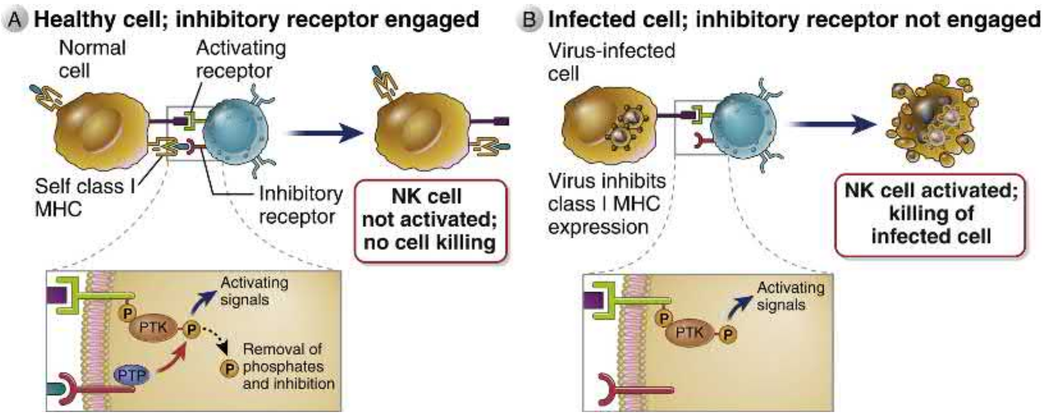

A Healthy cell; inhibitory receptor engaged
Normal cell
Activating receptor
Self class I MHC
Inhibitory receptor
NK cell not activated; no cell killing
Activating signals
P
PTK
P
Removal of phosphates and inhibition
P
PTP
B Infected cell; inhibitory receptor not engaged
Virus-infected cell
Virus inhibits class I MHC expression
NK cell activated; killing of infected cell
Activating signals
P
PTK
P

FIGURE 4.10 Functions of activating and inhibitory receptors of natural killer cells. **A,** Activating receptors of natural killer (NK) cells recognize ligands on target cells and activate protein tyrosine kinases *(PTKs),* whose activities are inhibited by inhibitory receptors that recognize class I major histocompatibility complex (MHC) molecules and activate protein tyrosine phosphatases *(PTP).* NK cells do not efficiently kill class I MHC–expressing healthy cells. **B,** If a viral infection or other stress inhibits class I MHC expression on infected cells and induces expression of additional activating ligands, the NK cell inhibitory receptor is not engaged and the activating receptor is unopposed to trigger responses of NK cells, including killing of target cells and cytokine secretion. In addition, infected cells or cancer cells may express increased amounts of activating ligands, inducing more tyrosine phosphorylation than can be removed by inhibitory receptor–associated phosphatases, resulting in killing of the stressed cells (not shown). Structural details and ligands of inhibitory and activating NK cell receptors are shown in Fig. 4.11.

Activating receptors on NK cells recognize a heterogeneous group of ligands, some of which may be expressed on normal cells and others mainly on cells that have undergone stress, are infected with microbes, or are neoplastic (Fig. 4.11) **.** Many of the NK cell–activating receptors are called killer cell Ig-like receptors (KIRs) because they contain a structural domain named the Ig fold, first identified in antibody (also known as Ig) molecules, discussed in Chapter 5. All proteins with Ig folds are members of the Ig superfamily. A second important group of activating NK receptors are similar to the family of C-type lectins, which are proteins with carbohydrate-binding properties similar to the CLRs discussed earlier in the chapter, although these NK receptors do not bind carbohydrates. One well-studied lectin-like NK cell–activating receptor is NKG2D, which binds class I MHC–like proteins, including MHC class I–related gene A (MIC-A) and MIC-B, and proteins of the ULBP family. The expression of all of these NKG2D ligands is increased by cellular stress, so they are found on virally infected cells and tumor cells but not normal cells. The NKG2D receptor associates with a signaling subunit named DAP10, which has signaling functions that stimulate NK cell cytotoxicity against target cells.

Another important activating receptor on NK cells is CD16 (FcγRIIIA), which is a low-affinity receptor for IgG antibodies. Antibody molecules have highly variable antigen-binding ends, and on the opposite end, they have an invariant portion, called the Fc region, that interacts with various other molecules in the immune system. We will describe the structure of antibodies in detail in Chapter 5, but for now, it is sufficient to know that CD16 binds to the Fc regions of certain types of antibodies called IgG1 and IgG3. CD16 associates with one of two different signaling proteins (FcεRIγ or ζ). During an infection, the adaptive immune system produces IgG1 and IgG3 antibodies that bind to microbial antigens expressed on the surface of infected cells, and CD16 on NK cells can bind to the Fc regions of these antibodies. As a result, CD16 generates activating signals, through its associated signaling partners, and the NK cells kill the infected cells that have been coated with antibody molecules. This process is called **antibody-dependent cell-mediated cytotoxicity;** it is an effector mechanism of adaptive immunity, which we will discuss in Chapter 13 when we consider humoral immunity.

Inhibitory receptors of NK cells recognize class I MHC molecules, which are cell surface proteins normally expressed on all healthy nucleated cells in the body (see Fig. 4.11) **.** A major function of class I MHC molecules, distinct from their role in regulating NK cell activation, is to display peptides derived from cytosolic proteins, including microbial proteins, on the cell surface for recognition by $CD8^+$ T lymphocytes. We will describe the structure and function of MHC molecules in relation to T cell antigen recognition in Chapter 6. For now, it is important to understand that NK cells use fundamentally different types of receptors than do T cells to recognize class I MHC molecules. These NK receptors for class I MHC molecules inhibit NK cell activation. This is useful because normal cells express class I MHC molecules, and many viruses and other causes of cell stress lead to a loss of cell surface expression of class I MHC. Thus, NK cells interpret the presence of class I MHC molecules as a marker of normal, healthy self, and their absence is an indication of infection or damage. As a result, NK cells will be inhibited by healthy cells but will not receive inhibitory signals from infected or stressed cells. At the same time, the NK cells are likely to receive activating signals from infected or damaged cells through activating receptors. The net result will be activation of the NK cells to secrete cytokines and to kill the infected or stressed cell. This ability of NK cells to become activated by host cells that lack class I MHC has been called recognition of missing self.

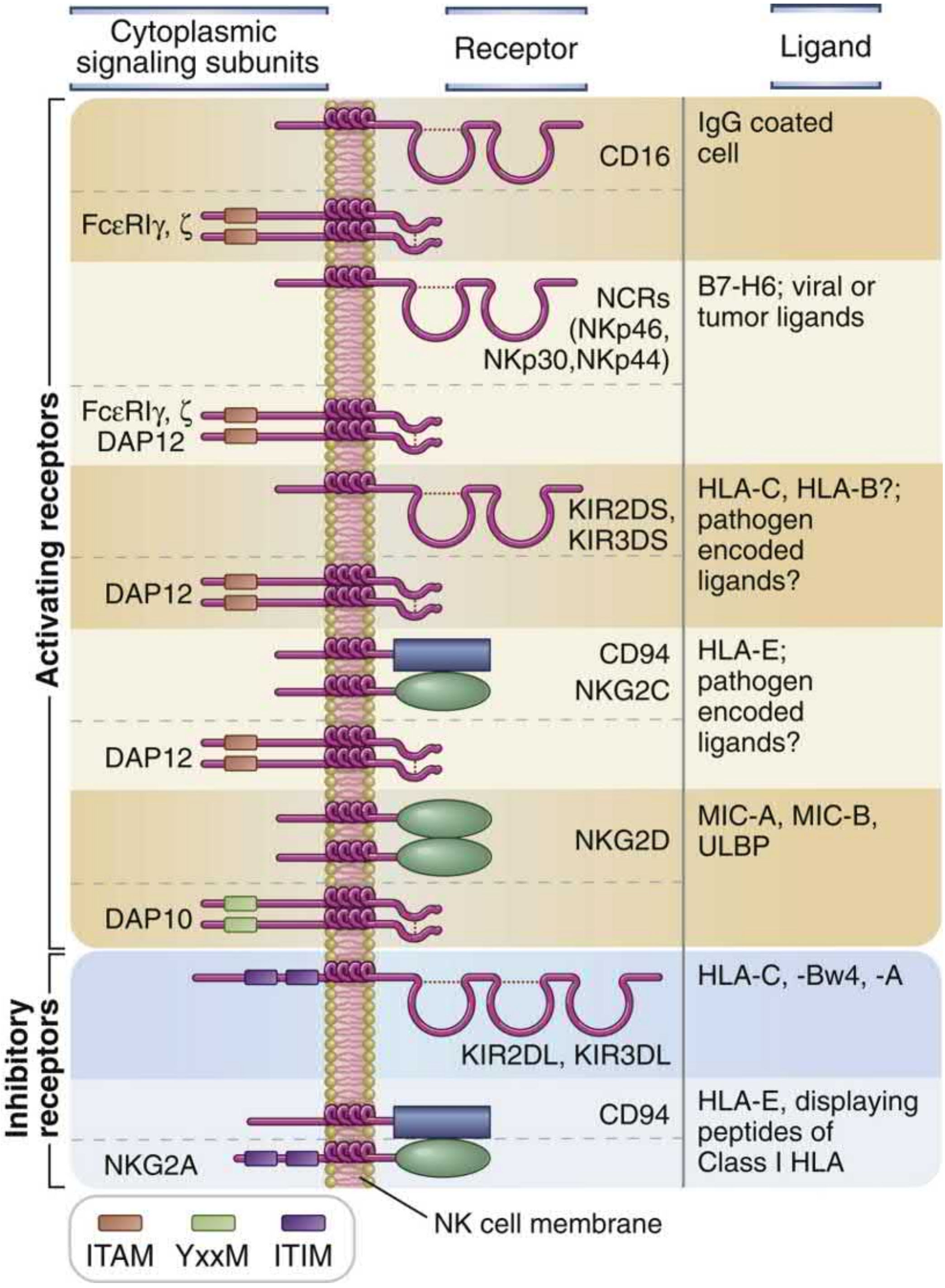

Cytoplasmic signaling subunits
Receptor
Ligand
Activating receptors
Inhibitory receptors
CD16
IgG coated cell
FcεRIγ, ζ
NCRs (NKp46, NKp30,NKp44)
B7-H6; viral or tumor ligands
FcεRIγ, ζ DAP12
KIR2DS, KIR3DS
HLA-C, HLA-B?; pathogen encoded ligands?
DAP12
CD94
NKG2C
HLA-E; pathogen encoded ligands?
DAP12
NKG2D
MIC-A, MIC-B, ULBP
DAP10
HLA-C, -Bw4, -A
KIR2DL, KIR3DL
CD94
HLA-E, displaying peptides of Class I HLA
NKG2A
NK cell membrane
ITAM
YxxM
ITIM

FIGURE 4.11 Structure and ligands of activating and inhibitory receptors of natural killer cells. Different activating and inhibitory receptors of NK cells use different associated signaling components and recognize different ligands. B7-H6 is a member of the B7 family that is expressed mainly on tumor

cells. *BAT3,* HLA-B-associated transcript 3; *HLA,* human leukocyte antigen; *ITAM,* immunoreceptor tyrosine-based activation motif; *HSPG,* heparan sulfate proteoglycan; *ITIM,* immunoreceptor tyrosine-based inhibition motif *KIR,* killer cell immunoglobulin *(Ig)*-like receptors; *MHC,* major histocompatibility complex; *MIC,* MHC class I polypeptide-related sequence; *NCR,* natural cytotoxicity receptor. *ULBP,* UL-16 binding protein.

The largest group of NK inhibitory receptors belong to the same KIR family that includes activating receptors, discussed earlier. These inhibitory KIRs bind a variety of different class I MHC molecules. Other inhibitory receptors are lectin-like, such as the CD94/NKG2A heterodimer, which recognizes a class I MHC molecule called HLA-E. Interestingly, HLA-E displays peptides derived from other class I MHC molecules, so CD94/NKG2A is a surveillance receptor for several different class I MHC molecules.

Activating and inhibitory NK receptors contain structural motifs in their cytoplasmic tails that engage the signaling pathways that respectively promote or inhibit target cell killing and cytokine secretion (see Figs. 4.10 and 4.11) . Activating receptors have **immunoreceptor tyrosine-based activation motifs (ITAMs)**, which contain tyrosine residues that become phosphorylated by cytoplasmic kinases after binding of ligands to the receptors. Other protein kinases are recruited to the modified ITAMs and become activated, and these kinases contribute to further signaling by phosphorylating additional proteins, eventually leading to cytotoxic activity and cytokine secretion. ITAMs also are found in the cytoplasmic tails of other signaling receptors in the immune system, including the antigen receptor complexes of T and B cells, and we will discuss their structure and signaling functions in more detail in Chapter 7. In some activating receptors, a single polypeptide chain contains the cytoplasmic ITAM and the extracellular ligand-binding portion. In other receptors, the ITAMs are in separate polypeptide chains, such as FcεRIγ (so named because it was first identified as a signaling chain of the Fcε receptor, see Chapter 20), ζ (a component of the TCR complex), and DAP12. These signaling proteins do not bind ligands but are noncovalently associated with the ligand-binding chains (see Fig. 4.11).

Inhibitory receptors of NK cells have **immunoreceptor tyrosine-based inhibition motifs** (ITIMs), which engage molecules that block the signaling pathways of activating receptors (see Figs. 4.10 and 4.11). ITIMs contain tyrosine residues that are phosphorylated when ligands bind to the inhibitory receptor, and then serve as docking sites for the recruitment and activation of tyrosine phosphatases, which remove phosphates from several signaling proteins generated by the signaling downstream of NK activating receptors. The end result is blocking of the signaling functions of activating receptors. ITIMs also are found in cytoplasmic tails of other receptors besides NK inhibitory receptors, and we will discuss their structure and signaling functions in more detail in Chapter 7.

KIR genes are polymorphic, meaning that there are several allelic variants in the human population. As a result, one person may express different receptors than another person. Groups of *KIR* alleles are often inherited together from a single parent. These groups of linked genes are called *KIR* haplotypes. There are two major *KIR* haplotypes and some rarer ones. Haplotypes differ in the number of receptors encoded, and some have more or fewer activating receptors than others. Some haplotypes are associated with increased susceptibility to some disorders, including spontaneous abortion and a type of eye inflammation called uveitis.

Cytokines can enhance the functional responses of NK cells. The major cytokines of the innate immune system that stimulate NK cell function are IL-12, IL-15, IL-18, and type I IFNs interferons (discussed later). Each of these cytokines enhances the cytotoxic activity of NK cells, and they can stimulate IFN-γ secretion by the NK cells independent of activating receptors. In addition, IL-15 is an important growth factor for NK cells.

T and B Lymphocytes With Limited Antigen Receptor Diversity

In contrast to the cells of the innate immune system that we have discussed so far, most T and B lymphocytes of the adaptive immune system use highly diverse receptors to recognize an enormous variety of different antigens. Certain small populations of lymphocytes express antigen receptors that are structurally the same as those of T and B cells, but these receptors have very little diversity. These T and B cell subsets may recognize structures expressed by many different or commonly encountered microbial species. T cells with limited antigen receptor diversity include invariant natural killer T (iNKT) cells, γδ T cells, mucosa-associated invariant T (MAIT) cells, and intraepithelial T cells with αβ TCRs (mentioned earlier). B cell subsets that produce antibodies with a limited set of specificities include B-1 cells and marginal-zone B cells. Although these T and B cells perform functions similar to those of their more clonally diverse counterparts, the nature of their specificities places them in a special category of lymphocytes that is more akin to cells of innate immunity than to cells of adaptive immunity. These special T and B cell subsets are described in Chapters 10 and 12, respectively.

Mast Cells

Mast cells are sentinel cells present in the skin, mucosal epithelium, and connective tissues that rapidly secrete proinflammatory cytokines and lipid mediators in response to infections and other stimuli. We introduced mast cells in Chapter 2. Recall that these cells contain abundant cytoplasmic granules filled with various inflammatory mediators that are released when the cells are activated, either by microbial products or by a special antibody-dependent mechanism. The granule contents include vasoactive amines (such as histamine) that cause vasodilation and increased capillary permeability, and proteolytic enzymes that can kill bacteria or inactivate microbial toxins. Mast cells also synthesize and secrete lipid mediators (such as leukotrienes and prostaglandins) and cytokines (such as TNF). Because mast cells are usually located adjacent to blood vessels (see Fig. 2.1B), their released granule contents rapidly induce changes in the blood vessels that promote acute inflammation. Mast cells express TLRs, and TLR ligands can induce mast cell degranulation. Mast cell–deficient mice are impaired in controlling bacterial infections, probably because of defective innate immune responses. Mast cell products also provide defense against helminths and are responsible for symptoms of allergic diseases. We will return to a detailed discussion of mast cells in the context of allergic diseases in Chapter 20.

Soluble Effector Molecules of Innate Immunity

Several different kinds of molecules that recognize microbes and promote innate responses exist in soluble form in the blood and extracellular fluids. These molecules provide early defense against pathogens that enter the circulation or are present outside host cells at some stage of their life cycle. The soluble effector molecules function in two major ways:

- By binding to microbes, they act as **opsonins** and enhance the ability of macrophages and neutrophils to phagocytose the microbes. This is because the phagocytic cells express membrane receptors specific for the opsonins, and these receptors can efficiently mediate the internalization of the complex of opsonin and bound microbes and subsequent destruction of the ingested microbes.
- After binding to microbes, soluble mediators of innate immunity promote inflammatory responses that bring more phagocytes to sites of infections and they may also directly kill microbes.

The soluble effector molecules are sometimes called the humoral branch of innate immunity, analogous to the humoral branch of adaptive immunity mediated by antibodies. The major components of the humoral innate immune system are the complement system, collectins, pentraxins, and ficolins, which are described next.

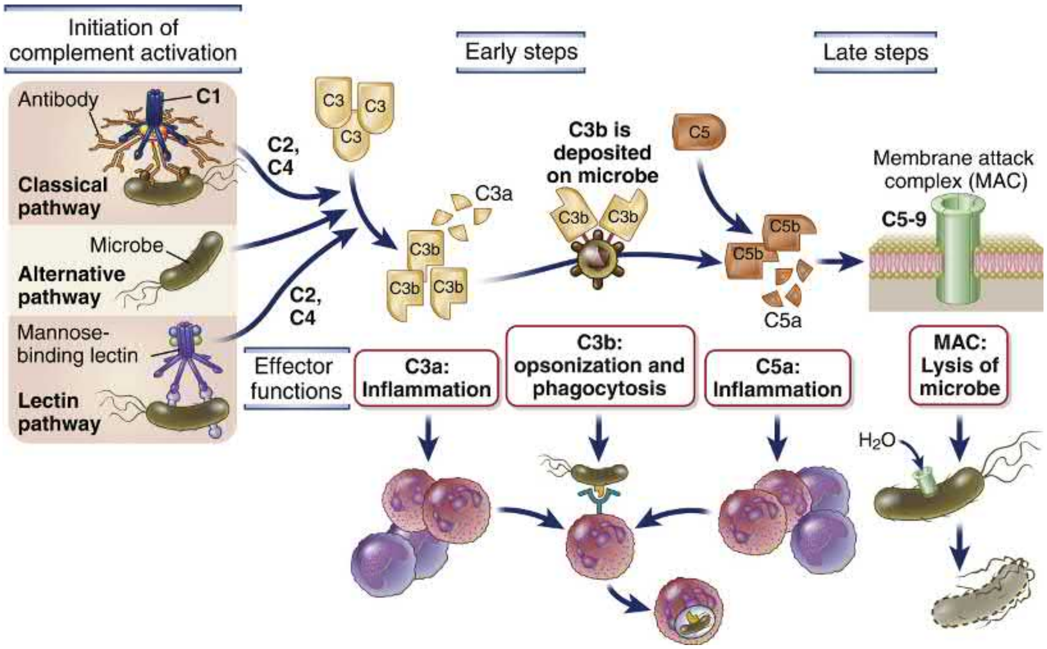

Initiation of complement activation
Early steps
Late steps
Antibody
C1
Classical pathway
Microbe
Alternative pathway
Mannose-binding lectin
Lectin pathway
C2, C4
C3
C3
C3
C2, C4
C3a
C3b
C3b
C3b
C3b is deposited on microbe
C3b
C3b
C5
C5b
C5b
C5a
Membrane attack complex (MAC)
C5-9
Effector functions
C3a: Inflammation
C3b: opsonization and phagocytosis
C5a: Inflammation
MAC: Lysis of microbe
H_2O

FIGURE 4.12 Pathways of complement activation.The activation of the complement system may be initiated by three distinct pathways, all of which lead to the production of C3a, which stimulates inflammation, and C3b (early steps). C3b initiates the late steps of complement activation, culminating in the production of peptides that also stimulate inflammation (C5a) and polymerized C9, which forms the membrane attack complex (late steps). The activation, functions, and regulation of the complement system are discussed in much more detail in Chapter 13.

The Complement System

The complement system consists of several plasma proteins that work together to opsonize microbes, to promote the recruitment of phagocytes to the site of infection, and in some cases to directly kill the microbes (Fig. 4.12) **.** Complement activation involves proteolytic cascades in which an inactive protein, called a zymogen, is altered to become an active protease that cleaves and thereby induces the proteolytic activity of the next complement protein in the cascade. Enzymatic cascades result in tremendous amplification of the amount of proteolytic products that are generated at each step. These products perform the effector functions of the complement system.

The first step in activation of the complement system is recognition of molecules on microbial surfaces, and this occurs in three ways, each referred to as a distinct pathway of complement activation.

- The **classical pathway,** so called because it was discovered first, uses a plasma protein called C1q to detect antibodies bound to the surface of microbes or other structures. Once C1q binds to the Fc portion of the antibodies, two associated serine proteases, called C1r and C1s, become active and initiate a proteolytic cascade involving other complement proteins. The classical pathway is one of the major effector mechanisms of the humoral arm of adaptive immune responses (see Chapter 13). Innate immune system soluble proteins called pentraxins, which are discussed later, can also bind C1q and initiate the classical pathway.
- The **alternative pathway**, which was discovered later but is phylogenetically older than the classical pathway, is triggered when a complement protein called C3 directly recognizes certain microbial surface structures, such as bacterial LPS. C3 is also constitutively activated at a low level in blood and extravascular fluid and binds to cell surfaces, but it is then inhibited by regulatory molecules

present on mammalian cells. Because microbes lack these regulatory proteins, the spontaneous activation can be amplified on microbial surfaces. Thus, this pathway can distinguish normal self from foreign microbes on the basis of the presence or absence of the regulatory proteins.

- The **lectin pathway** is triggered by a plasma protein called mannose-binding lectin (MBL), which recognizes terminal mannose residues on microbial glycoproteins and glycolipids, similar to the mannose receptor on phagocytes described earlier. MBL is a member of the collectin family (discussed later) with a hexameric structure similar to that of the C1q component of the complement system. After MBL binds to microbes, two zymogens called MASP1 (mannose-associated serine protease 1 or mannan-binding lectin-associated serine protease) and MASP2, with functions similar to those of C1r and C1s, associate with MBL and initiate downstream proteolytic steps identical to the classical pathway.

Recognition of microbes by any of the three complement pathways results in sequential recruitment and assembly of additional complement proteins into protease complexes (see Fig. 4.12) **.** One of these complexes, called C3 convertase, cleaves the central protein of the complement system, C3, producing C3a and C3b. The larger C3b fragment becomes covalently attached to the microbial surface where the complement pathway was activated. The sequential enzymatic activity of complement proteins provides such tremendous amplification that millions of C3b molecules can deposit on the surface of a microbe within 2 or 3 minutes. C3b serves as an opsonin to promote phagocytosis of the microbes. The smaller fragment, C3a, is released and stimulates inflammation by acting as a chemoattractant for neutrophils, by inducing mast cell degranulation, and by directly increasing vascular permeability so that plasma proteins and fluid leak into sites of infections. C3b binds other complement proteins to form a protease called C5 convertase that cleaves C5, generating a released peptide (C5a) and a larger fragment (C5b) that remains attached to the microbial cell membranes. C5a exerts the same proinflammatory effects as C3a and is more potent. C5b initiates the formation of a complex of the complement proteins C6, C7, C8, and C9, which are assembled into a membrane pore called the **membrane attack complex** (MAC) that causes lysis of the cells where complement is activated.

The complement system, activated by the alternative and lectin pathways, is an essential component of innate immunity, and patients with deficiencies in C3 are highly susceptible to recurrent, often lethal, bacterial infections. Genetic deficiencies in MAC formation (the terminal product of the classical pathway) cause increased susceptibility to only a limited number of microbes, notably *Neisseria* bacteria, which have thin cell walls that make them especially susceptible to the lytic action of the MAC. The complement system also contributes to cell and tissue injury in many inflammatory and autoimmune diseases. We will discuss the complement system in more detail in Chapter 13.

Pentraxins

Several plasma proteins that recognize microbial structures and participate in innate immunity belong to the pentraxin family, which is a phylogenetically old group of structurally homologous pentameric proteins. Prominent members of this family include the short pentraxins, C-reactive protein (CRP) and serum amyloid P (SAP), and the long pentraxin PTX3. Both CRP and SAP bind to several different species of bacteria and fungi. The molecular ligands recognized by CRP and SAP include phosphorylcholine and phosphatidylethanolamine, respectively, which are found on bacterial membranes and become exposed on apoptotic cells. CRP, SAP, and PTX3 all activate complement by binding C1q and initiating the classical pathway.

Plasma concentrations of CRP are very low in healthy individuals but can increase up to 1000-fold during infections and in response to other inflammatory stimuli. The increased CRP levels result from increased synthesis by liver cells induced by the cytokines IL-6, IL-

1, and TNF, which are produced by phagocytes and DCs as part of the innate immune response to infections or injury. Liver synthesis and plasma levels of several other proteins, including SAP and some unrelated to the pentraxins, also increase in response to IL-1, IL-6, and TNF. All of these plasma proteins are called **acute-phase proteins** because they are elevated in the blood during acute inflammatory reactions, and their increased production is part of the **acute-phase response** to infection and other insults.

PTX3 is produced by several cell types, including DCs, macrophages, and endothelial cells, in response to TLR ligands and inflammatory cytokines, such as TNF, and may be considered an acute-phase reactant. PTX3 is also stored in neutrophil granules and released as neutrophils die. PTX3 recognizes various molecules on fungi, certain bacteria, and viruses, as well as apoptotic cells, and activates the classical complement pathway. Studies with knockout mice reveal that PTX3 provides protection against these microbes, including the fungus *Aspergillus fumigatus* and the influenza virus.

Collectins and Ficolins

The **collectins** are a family of trimeric or hexameric proteins, each subunit of which contains a collagen-like tail connected by a neck region to a calcium-dependent (C-type) lectin head. Three members of this family serve as soluble effector molecules in the innate immune system; these are MBL and pulmonary surfactant proteins SP-A and SP-D.

MBL, which is a soluble pattern recognition receptor that binds carbohydrates with terminal mannose and fucose, was discussed earlier in relation to the lectin pathway of complement activation (Fig. 4.13). MBL also functions as an opsonin by binding to and enhancing phagocytosis of microbes. Recall that opsonins simultaneously bind microbes and a surface receptor on phagocyte membranes, and in the case of MBL, the surface receptor is called the C1q receptor because it also binds C1q. This receptor mediates the internalization of microbes that are opsonized by MBL. The gene encoding MBL is polymorphic, and certain alleles are associated with impaired hexamer formation and reduced blood levels. Low MBL levels result in increased susceptibility to a variety of infections, especially in individuals who have other immune defects.

Surfactant protein A (SP-A) and surfactant protein D (SP-D) are collectins with lipophilic properties shared by other surfactants. They are found in the alveoli of the lungs, and their major functions are to maintain the ability of alveoli to expand upon inhalation by reducing surface tension of alveolar fluid, and as mediators of innate immune responses in the lung. They bind to various microorganisms and act as opsonins, facilitating ingestion by alveolar macrophages. SP-A and SP-D can also directly inhibit bacterial growth, and they may activate macrophages. SP-A– and SP-D–deficient mice have impaired abilities to resist a variety of pulmonary infections.

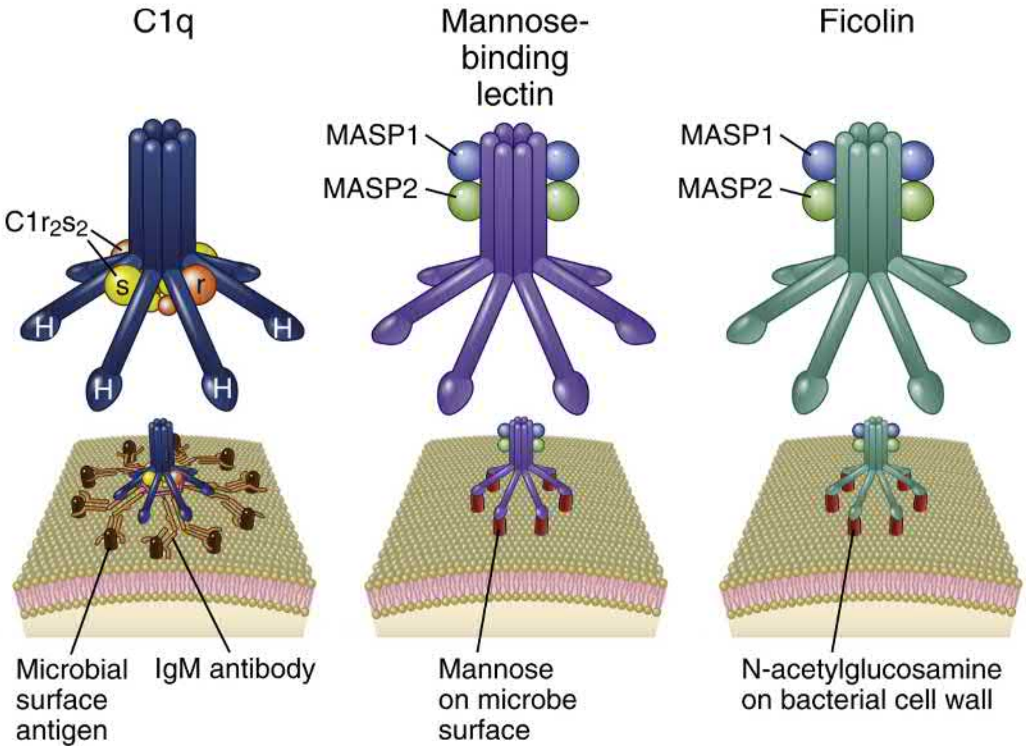

C1q
Mannose-binding lectin
Ficolin
C1r2s2
s
r
H
H
H
H
MASP1
MASP2
MASP1
MASP2
Microbial surface antigen
IgM antibody
Mannose on microbe surface
N-acetylglucosamine on bacterial cell wall

FIGURE 4.13 C1, mannose-binding lectin, and ficolin. These three homologous hexameric proteins can all initiate complement activation on binding to their ligands on cell surfaces. C-type lectin–like globular heads *(H)* at the end of collagenous-like stalks in the C1q and mannose-binding lectin proteins bind the Fc regions of immunoglobulin M *(IgM)* or mannose on the surface of microbes, respectively. Fibrinogen-like globular heads on ficolin bind *N*-acetylglucosamine on the surface of microbes. Binding results in conformational changes that activate the serine protease activity of C1r and C1s, associated with C1q, or mannose-associated serine protease 1 *(MASP1)* and *MASP2,* associated with mannose-binding lectin and ficolin.

Ficolins are plasma proteins that are structurally similar to collectins. They possess a collagen-like domain, but instead of a C-type lectin domain, they have a fibrinogen-type carbohydrate recognition domain (see Fig. 4.13). Ficolins have been shown to bind several species of bacteria, opsonizing them and activating complement in a manner similar to that of MBL. The ligands of the ficolins include *N*-acetylglucosamine and the lipoteichoic acid component of the cell walls of gram-positive bacteria.

Now that we have discussed the general properties and various components of the innate immune system, including the cells, cellular pathogen recognition receptors, and soluble effector molecules, we can consider how these various components work to protect against pathogens. The three major mechanisms by which the innate immune system protects against infections are by inducing inflammation, inducing antiviral defense, and stimulating adaptive immunity.

The Inflammatory Response

The principal way by which the innate immune system deals with infections and tissue injury is to stimulate acute inflammation, which is the accumulation of leukocytes, plasma proteins, and fluid derived from the blood at an extravascular tissue site of infection or injury (Fig. 4.14) . Leukocytes and plasma proteins, which are critical for innate defense against microbes, normally circulate in the blood and must be recruited to extravascular sites of infection and injury, where they perform the effector functions that kill microbes and begin to repair damaged tissue. Acute inflammatory responses begin with recognition of microbial PAMPs or DAMPs from injured host cells by tissue sentinel cells, mainly macrophages, DCs, and mast cells. These sentinels respond by secreting mediators that act on small blood vessels in ways that promote increased blood flow, delivery of plasma proteins, and migration of leukocytes into the tissues. Detailed descriptions of the various mediators and pathologic manifestations of inflammation can be found in pathology textbooks. We will focus our discussion on particular aspects of the inflammatory process that have broad relevance to both innate and adaptive immunity and immune-mediated inflammatory diseases, beginning with a description of the important cytokines in innate immune inflammatory responses.

The Major Proinflammatory Cytokines of Innate Immunity

One of the earliest responses of the innate immune system to infection and tissue damage is the secretion of cytokines by tissue cells, which is critical for the acute inflammatory response. The cytokines of innate immunity have some important general properties and functions (Table 4.5):

- They are produced mainly by tissue macrophages and DCs, although other cell types, including mast cells, endothelial cells, and some epithelial cells, can also produce them.
- Most of these cytokines act on cells close to their cell of origin (paracrine action). In some severe infections, enough of the cytokines may be produced so that significant amounts enter the circulation and act at a distance (endocrine action).

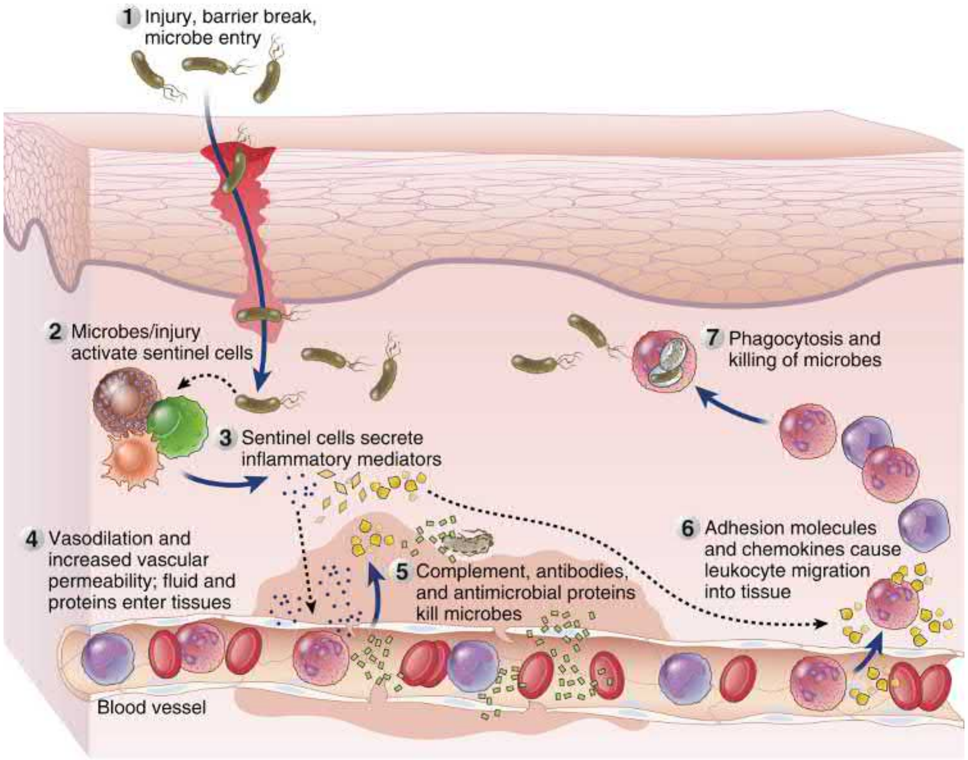

1 Injury, barrier break, microbe entry
2 Microbes/injury activate sentinel cells
3 Sentinel cells secrete inflammatory mediators
4 Vasodilation and increased vascular permeability; fluid and proteins enter tissues
5 Complement, antibodies, and antimicrobial proteins kill microbes
6 Adhesion molecules and chemokines cause leukocyte migration into tissue
7 Phagocytosis and killing of microbes
Blood vessel

FIGURE 4.14 Acute inflammatory response.Acute inflammatory responses begin when microbes transgress epithelial barriers or when tissue is injured *(1),* and then pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) activate sentinel cells, such as macrophages, dendritic cells, mast cells *(2),* to secrete cytokines and other mediators *(3).* Some of these mediators (e.g., histamine, prostaglandins) increase the permeability of capillaries *(4),* leading to the entry of plasma proteins (e.g., complement proteins) into the tissues *(5),* and others (interleukin-1, tumor necrosis factor) increase expression of endothelial adhesion molecules and chemokines that promote the movement of leukocytes from the post-capillary venules into the tissues *(6),* where the leukocytes

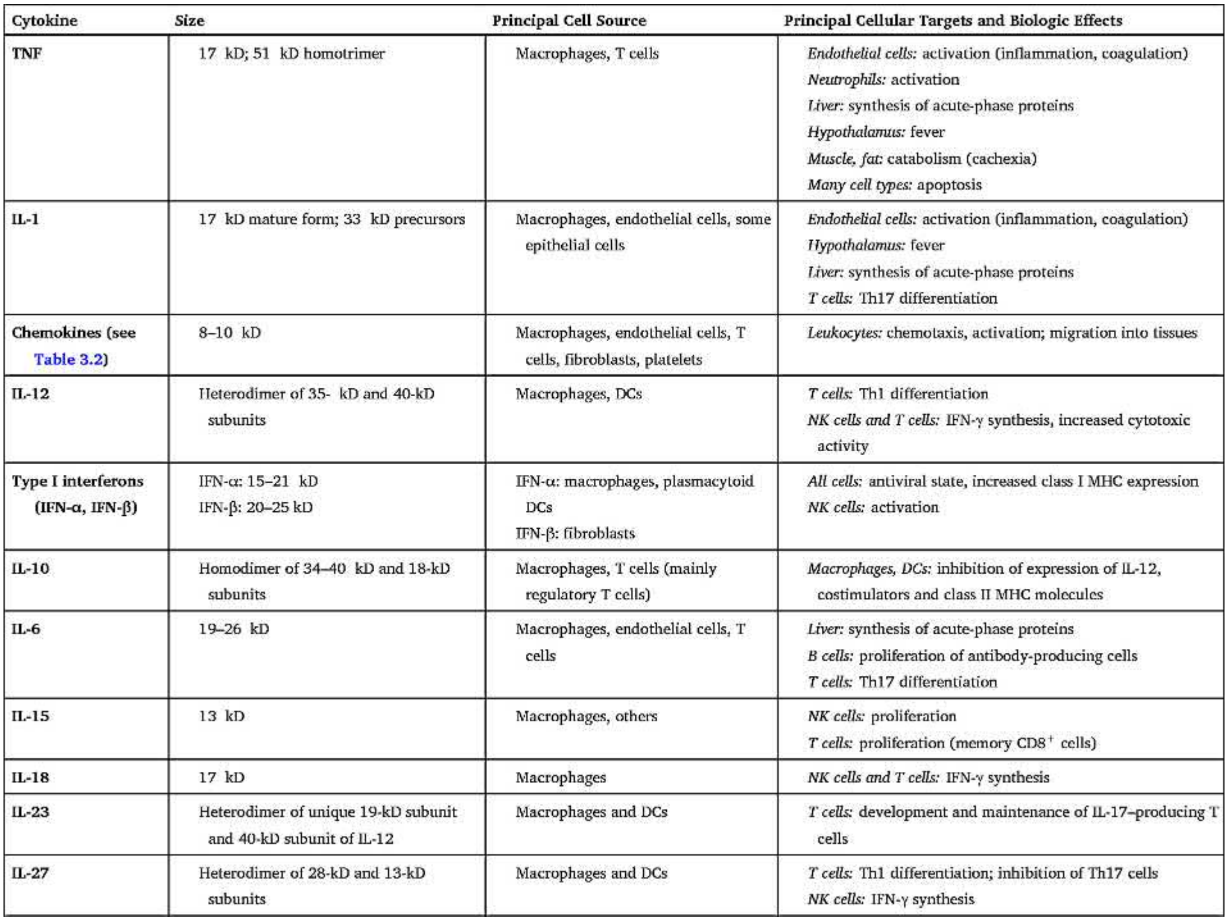
destroy microbes, clear damaged cells *(7)*, and promote more inflammation and repair.

- Different cytokines have similar or overlapping actions or are functionally unique. One cytokine may stimulate the production of others, thus setting up cascades that amplify the reaction or induce new reactions.
- The cytokines of innate immunity serve several roles: inducing inflammation, inhibiting viral replication, promoting T cell responses, and limiting innate immune responses. These functions are described next and later in the chapter.
- Many cytokines that are produced by innate immune cells, such as TNF, IL-17, IL-5, and IFN-γ, are also produced by T lymphocytes in adaptive immune responses.
- Three of the most important proinflammatory cytokines of the innate immune system are TNF, IL-1 (both of which we have mentioned several times), and IL-6. We will discuss the major features of these cytokines, focusing mainly on TNF and IL-1, before describing their role in acute inflammation.

Tumor Necrosis Factor

TNF is a mediator of the acute inflammatory response to bacteria and other infectious microbes. The name of this cytokine derives from its original identification as a serum substance (factor) that caused necrosis of tumors, now known to be the result of inflammation and thrombosis of tumor blood vessels. TNF is also called TNF-α to distinguish it from the closely related TNF-β, which is also called lymphotoxin. TNF is produced mainly by macrophages, and also by other cell types, including DCs and mast cells. In macrophages, it is synthesized as a nonglycosylated type II membrane homotrimeric protein that is able to bind to one form of TNF receptor. The membrane form of TNF is cleaved by a membrane-associated metalloproteinase, releasing a polypeptide fragment, and three of these polypeptide chains polymerize to form a triangular pyramid-shaped circulating TNF protein (Fig. 4.15). The receptor-binding sites are at the base of the pyramid, allowing simultaneous binding of the cytokine to three receptor molecules. TNF is a member of a large family of homologous proteins called the TNF superfamily, all of which share the feature of forming homotrimers (see Appendix II).

TABLE 4.5



Cytokine	Size	Principal Cell Source	Principal Cellular Targets and Biologic Effects
TNF	17 kD; 51 kD homotrimer	Macrophages, T cells	*Endothelial cells:* activation (inflammation, coagulation) *Neutrophils:* activation *Liver:* synthesis of acute-phase proteins *Hypothalamus:* fever *Muscle, fat:* catabolism (cachexia) *Many cell types:* apoptosis
IL-1	17 kD mature form; 33 kD precursors	Macrophages, endothelial cells, some epithelial cells	*Endothelial cells:* activation (inflammation, coagulation) *Hypothalamus:* fever *Liver:* synthesis of acute-phase proteins *T cells:* Th17 differentiation
Chemokines (see Table 3.2)	8–10 kD	Macrophages, endothelial cells, T cells, fibroblasts, platelets	*Leukocytes:* chemotaxis, activation; migration into tissues
IL-12	Heterodimer of 35- kD and 40-kD subunits	Macrophages, DCs	*T cells:* Th1 differentiation *NK cells and T cells:* IFN-γ synthesis, increased cytotoxic activity
Type I interferons (IFN-α, IFN-β)	IFN-α: 15–21 kD IFN-β: 20–25 kD	IFN-α: macrophages, plasmacytoid DCs IFN-β: fibroblasts	*All cells:* antiviral state, increased class I MHC expression *NK cells:* activation
IL-10	Homodimer of 34–40 kD and 18-kD subunits	Macrophages, T cells (mainly regulatory T cells)	*Macrophages, DCs:* inhibition of expression of IL-12, costimulators and class II MHC molecules
IL-6	19–26 kD	Macrophages, endothelial cells, T cells	*Liver:* synthesis of acute-phase proteins *B cells:* proliferation of antibody-producing cells *T cells:* Th17 differentiation
IL-15	13 kD	Macrophages, others	*NK cells:* proliferation *T cells:* proliferation (memory CD8$^+$ cells)
IL-18	17 kD	Macrophages	*NK cells and T cells:* IFN-γ synthesis
IL-23	Heterodimer of unique 19-kD subunit and 40-kD subunit of IL-12	Macrophages and DCs	*T cells:* development and maintenance of IL-17–producing T cells
IL-27	Heterodimer of 28-kD and 13-kD subunits	Macrophages and DCs	*T cells:* Th1 differentiation; inhibition of Th17 cells *NK cells:* IFN-γ synthesis

DC, Dendritic cells; *MHC,* major histocompatibility complex; *IFN,* interferon; *IL,* interleukin; *TNF,* tumor necrosis factor. (Also see Appendix I.)

There are two distinct TNF receptors called type I (TNFRI) and type II (TNFRII). The affinities of TNF for its receptors are unusually low for a cytokine, the K_d being only approximately 1×10^{-9} M for binding to TNFRI and approximately 5×10^{-10} M for binding to TNFRII. Both TNF receptors are present on most cell types. The TNF receptors are members of a large family of proteins called the TNF receptor superfamily, many of which are involved in immune and inflammatory responses. These receptors exist as trimers in the plasma membrane. Ligand binding to some TNF receptor family members, such as TNFRI, TNFRII, and CD40, leads to the recruitment of proteins called TNF receptor–associated factors (TRAFs) to the cytoplasmic domains of the receptors. The TRAFs activate transcription factors, notably NF-κB and AP1 (see Chapter 7). Cytokine binding to some family members, such as TNFRI, may lead to recruitment of an adaptor protein that activates caspases and triggers apoptosis. Thus, different members of the TNF receptor family can induce gene expression or cell death, and some can do both.

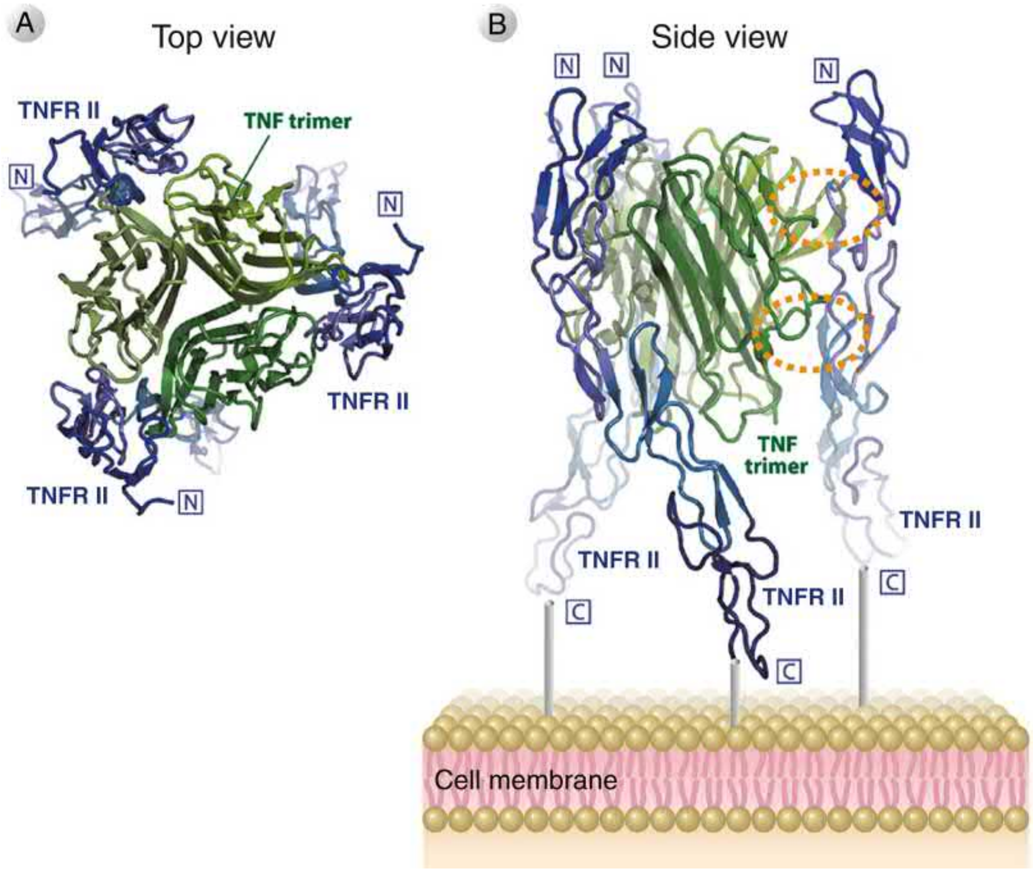

A
Top view
TNFR II
TNF trimer
N
N
TNFR II
TNFR II
N
B
Side view
N
N
N
TNF trimer
TNFR II
TNFR II
TNFR II
C
C
C
Cell membrane

FIGURE 4.15 Structure of the tumor necrosis factor (TNF) receptor with bound TNF.The ribbon structure depicts a *top view* **(A)** and a *side view* **(B)** of a complex of three type II TNF receptors *(TNFR II)* and one molecule of bound trimeric TNF, revealed by x-ray crystallography. The three TNFR II molecules, colored *blue,* together bind one homotrimer of TNF, colored *green,* with each receptor molecule interacting with two different TNF monomers in the homotrimer complex. The binding regions of one of the three TNFR II molecules to two TNF monomers are highlighted in the side view by *orange ovals.*

Modified from Mukai Y, Nakamura T, Yoshikawa M, et al. Solution of the structure of the TNF-TNFR2 complex. *Sci Signal.* 2010;3:ra83.

TNF production by macrophages is stimulated by PAMPs and DAMPs. TLRs, NLRs, RLRs, and CDSs can all induce TNF gene expression, in part by activation of the NF-κB transcription factor. Many different microbial products can therefore induce TNF production. TNF has multiple local and systemic effects that account for many of the reactions in inflammation (Fig. 4.16). TNF is a major contributor to inflammation in several chronic inflammatory diseases, and anti-TNF agents have become the mainstay of treatment of many of these diseases. Large amounts of this cytokine may be produced during infections with gram-negative and gram-positive bacteria, which express and release the cell wall TLR ligands LPS and lipoteichoic acid, respectively. Septic shock, a life-threatening condition resulting from severe infections, is mediated in large part by TNF. We will discuss septic shock later in this chapter.

Interleukin-1

IL-1 is also a mediator of the acute inflammatory response and has many actions similar to those of TNF. A major cellular source of IL-1, like that of TNF, is activated mononuclear phagocytes. IL-1 is also produced by many cell types other than macrophages, such as neutrophils, DCs, epithelial cells (e.g., keratinocytes), and endothelial cells. There are two forms of IL-1, called IL-1α and IL-1β, that are less than 30% homologous to each other, but they bind to the same cell surface receptors and have the same biologic activities. The main biologically active secreted form in the setting of infections and most immune responses is IL-1β.

IL-1 production usually requires two distinct signals, one that activates new gene transcription and production of a 33-kD precursor pro–IL-1β polypeptide and a second that activates the inflammasome to proteolytically cleave the precursor to generate the 17-kD mature IL-1β protein (see Fig. 4.6). As discussed earlier in this chapter, IL-1β gene transcription is induced by TLR, NLR, and RLR signaling pathways that activate NF-κB, whereas pro–IL-1β cleavage is mediated by caspase-1, which is activated by inflammasomes. TNF can also stimulate phagocytes and other cell types to produce IL-1. This is an example of a cascade of cytokines that have similar biologic activities. Unlike most secreted proteins, neither IL-1α nor IL-1β has a hydrophobic signal sequence to target the nascent polypeptide to the endoplasmic reticulum membrane. As discussed earlier, IL-1β may be secreted through membrane pores formed by gasdermin D.

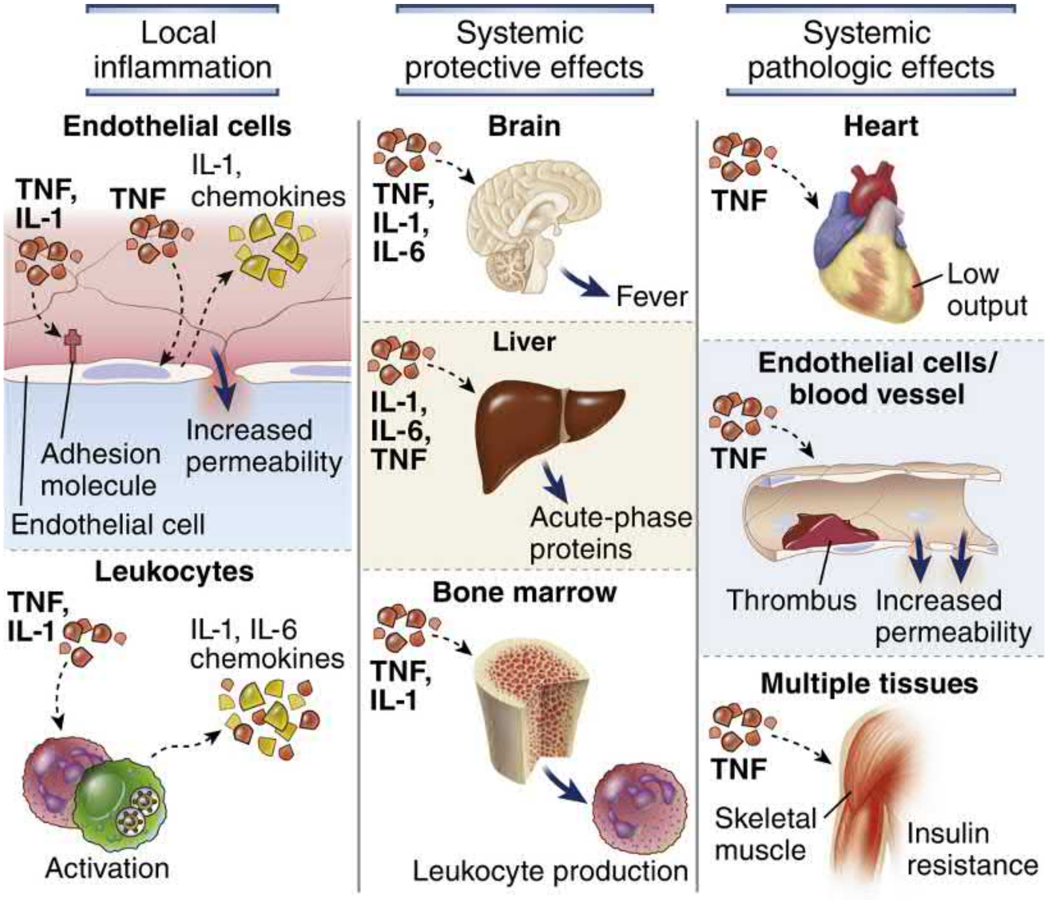

Local inflammation
Systemic protective effects
Systemic pathologic effects
Endothelial cells
TNF, IL-1
TNF
IL-1, chemokines
Adhesion molecule
Increased permeability
Endothelial cell
Leukocytes
TNF, IL-1
IL-1, IL-6 chemokines
Activation
Brain
TNF, IL-1, IL-6
Fever
Liver
IL-1, IL-6, TNF
Acute-phase proteins
Bone marrow
TNF, IL-1
Leukocyte production
Heart
TNF
Low output
Endothelial cells/ blood vessel
TNF
Thrombus
Increased permeability
Multiple tissues
TNF
Skeletal muscle
Insulin resistance

FIGURE 4.16 Local and systemic actions of cytokines in inflammation. Tumor necrosis factor *(TNF),* interleukin-1 *(IL-1),* and IL-6 have multiple local and systemic inflammatory effects. TNF and IL-1 act on leukocytes and endothelium to induce acute inflammation, and both cytokines induce the expression of IL-6 from leukocytes and other cell types. TNF, IL-1, and IL-6 mediate protective systemic effects of inflammation, including induction of fever, acute-phase protein synthesis by the liver, and increased production of leukocytes by the bone marrow. Systemic TNF can cause the pathologic abnormalities that lead to septic shock, including decreased cardiac function, thrombosis, capillary leak, and metabolic abnormalities due to insulin resistance.

IL-1 mediates its biologic effects through a membrane receptor called the type I IL-1 receptor, which is expressed on many cell types, including endothelial cells, epithelial cells, and leukocytes. This receptor is an integral membrane protein that contains an extracellular ligand-binding Ig domain and a TIR signaling domain in the cytosolic region, which we described earlier in reference to TLRs. The signaling events that occur when IL-1 binds to the type I IL-1 receptor are similar to those triggered by TLRs and result in the activation of NF-κB and AP1 (activator protein 1) transcription factors (see Chapter 7). A second IL-1 receptor, called the type II IL-1 receptor, appears incapable of activating downstream signals, and serves as a decoy receptor that limits responses to IL-1.

Interleukin-6

IL-6 is another important cytokine in acute inflammatory responses that has both local and systemic effects. It induces the synthesis of acute-phase reactants by the liver and promotes the differentiation of IL-17–producing helper T cells. IL-6 is synthesized by mononuclear phagocytes, DCs, vascular endothelial cells, fibroblasts, and other cells in response to PAMPs and DAMPs and in response to IL-1 and TNF. IL-6 is a homodimer that belongs to the type I cytokine family (see Chapter 7). The receptor for IL-6 consists of a cytokine-binding polypeptide chain and a signal-transducing subunit (called gp130) that is also the signaling component of receptors for other cytokines. The gp130 subunit is widely expressed on many cell types, but the IL-6 binding chain is only expressed by leukocytes and hepatocytes, as a transmembrane protein in association with gp130. However, a soluble form of the IL-6 binding chain is generated by proteolytic cleavage of the membrane form and is present in blood and tissue fluids. This soluble form can bind IL-6, and then the complex can associate with the extracellular part of gp130 on many cell types and initiate signaling. This mechanism is called trans-signaling. The IL-6 receptor engages a signaling pathway that activates the transcription factor STAT3 (see Chapter 7). IL-6 is a major contributor to inflammation in several human inflammatory diseases, including rheumatoid arthritis, and antibodies specific for the IL-6 receptor are used to treat some forms of arthritis. Some lymphoproliferative disorders such as Castleman's disease are caused by human herpesvirus-8 (HHV-8), a virus that encodes a homolog of IL-6, and IL-6 blockade has been used to treat these diseases.

Other Cytokines Produced During Innate Immune Responses

In addition to TNF, IL-1, and IL-6, DCs and macrophages activated by PAMPs and DAMPs produce other cytokines that have important roles in innate immune responses (see Table 4.5). We will discuss the main features of some of these cytokines and their roles in innate immunity in this section; interferons and inhibitory cytokines are discussed later in the chapter.

IL-12 is secreted by DCs and macrophages and stimulates IFN-γ production by ILC1s, NK cells, and T cells; enhances NK cell– and CTL-mediated cytotoxicity; and promotes differentiation of Th1 cells. IL-12 exists as a disulfide-linked heterodimer of 35-kD (p35) and 40-kD (p40) subunits. The p35 subunit is a member of the type I cytokine family, and the p40 subunit is also a component of the cytokine IL-23, which is involved in the differentiation of Th17 cells. Therefore, an antibody specific for p40 blocks both IL-12 and IL-23 and thus inhibits the IL-12–dependent development of Th1 cells and the IL-23–dependent development of Th17 cells. This antibody is approved for the treatment of inflammatory bowel disease and psoriasis, which are caused by Th1 and/or Th17 cytokines.

The principal sources of IL-12 are activated DCs and macrophages. Many cells appear to synthesize the p35 subunit, but macrophages and DCs are the main cell types that produce the p40 component and therefore the biologically active cytokine. During innate immune reactions to microbes, IL-12 is produced in response to TLR and other pattern recognition receptor signaling induced by many microbial stimuli, including bacterial LPS or lipoteichoic acid and virus infections. IFN-γ produced by NK cells or T cells also stimulates IL-12 production, contributing to a positive feedback loop.

The receptor for IL-12 is a heterodimer composed of β1 and β2 subunits, both of which are members of the type I cytokine receptor family. Both chains are required for high-affinity binding of IL-12 and for signaling, which activates the transcription factor STAT4. Expression of the β2 chain of the IL-12 receptor is itself enhanced by IFN-γ, whose production is stimulated by IL-12. This is an example of a positive amplification loop in immune responses. Studies with gene knockout mice and the phenotype of rare patients with mutations in the IL-12 receptor support the conclusion that IL-12 is important for IFN-γ production by NK cells and T cells and for host resistance to intracellular bacteria and some viruses. For example, patients with mutations in the IL-12 receptor β1 subunit have been described, and they are highly susceptible to infections with intracellular bacteria, notably *Salmonella* and mycobacteria. IL-12 secreted by DCs during antigen presentation to naive CD4$^+$ T cells promotes the differentiation of these cells into the Th1 subset of helper T cells, which are important for defense against intracellular infections (see Chapter 10). This is a key way in which innate immunity shapes adaptive immune responses.

IL-18 enhances the functions of NK cells, similar to IL-12. Recall that the production of IL-18, like that of IL-1, is dependent on inflammasomes. Also like IL-1, IL-18 binds to a receptor that signals through a TIR domain. Children with gain-of-function mutations in *NLRC4* have very high levels of inflammasome-generated IL-18 produced by intestinal epithelial cells and suffer from a systemic macrophage activation syndrome, likely due to excessive IL-18–driven production of IFN-γ from NK cells.

IL-15 stimulates growth and functions of ILC1s, NK cells, and some T cells. IL-15 is structurally homologous to the T cell growth factor IL-2, and the heterotrimeric IL-15 receptor shares two subunits with the IL-2 receptor. An interesting feature of IL-15 is that it can be expressed on the cell surface bound to the *α* chain of its receptor and in this form can be presented to and stimulate nearby cells that express a receptor composed of the other two chains (β and γ). IL-15 presented this way by DCs to NK cells in lymph nodes activates signaling pathways that promote NK cell IFN-γ production. IL-15 also serves as a survival factor for NK and memory CD8$^+$ T cells.

IL-25, thymic stromal lymphopoietin (TSLP), and IL-33 are structurally unrelated cytokines produced by epithelial barrier cells, as well as other cell types, which stimulate ILC2s, Th2 cells, and mast cells to produce IL-4, IL-5, and IL-13. The latter cytokines are important for defense against helminths, but also contribute to allergic disease (see Chapter 20). IL-33 is constitutively expressed by barrier epithelial cells and stored in their nuclei. It is often called an alarmin because it is rapidly released from damaged epithelial cells and then stimulates innate and adaptive responses.

In addition to the cytokines discussed here, other cytokines play important roles in both innate and adaptive immune responses, including IL-5, IL-17, and IFN-γ. These cytokines will be discussed in detail in Chapter 10, when we consider helper T cell subsets that produce them.

Sequence of Events in Inflammation: Vascular Changes and Leukocyte Migration Into Tissues

Acute inflammatory responses are initiated when sentinel cells, including mast cells, tissue-resident macrophages, and DCs, which are present in normal tissues before infection, use TLRs and cytosolic innate pattern recognition receptors to sense microbes and injured cells (see Fig 4.14) **.** Mast cells respond to PAMPs and DAMPs by secreting histamine and prostaglandins that cause vasodilation and increased capillary permeability, which increases blood flow through tissues and increases movement of plasma proteins such as complement proteins, pentraxins, collectins, and antibodies out of the blood vessels. These soluble innate effector molecules work in tandem with leukocytes that are recruited into tissue from the circulation.

Recruitment of large numbers of neutrophils, followed by monocytes, from blood into tissues typically occurs as part of the acute inflammatory response to infections and tissue injury. The vasodilation caused by mast cell–derived histamine and prostaglandins increases the number of these leukocytes entering affected tissues and also changes blood flow characteristics that enhance physical interactions of circulating leukocytes with blood vessel walls. TNF, IL-1, IL-6, and chemokines, all of which are produced by sentinel cells at the sites of infection or tissue injury, have multiple effects on capillaries, venules, leukocytes, and bone marrow, which together increase the local delivery of cells that can fight infections and repair tissues (see Figs. 4.14 and 4.16; see also Fig. 3.3). Leukocyte recruitment was described in Chapter 3 and will be considered only briefly here.

Postcapillary venule endothelial cells increase surface expression of adhesion molecules for leukocytes. Expression of E-selectin and ligands for integrins, including intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are induced by TNF and IL-1 activation of transcription factors, including NF-κB. P-selectin stored in endothelial cell cytoplasmic granules may be mobilized to the cell surface in response to thrombin generated by the coagulation cascade.

TNF and IL-1 also stimulate various cells to secrete chemokines, such as CXCL8 and CCL2, which bind to receptors on neutrophils and monocytes, respectively. As discussed in Chapter 3, these chemokines increase the affinity of leukocyte integrins for their ligands and stimulate directional movement of leukocytes. The result of increased selectin, integrin, and chemokine expression is an increase in neutrophil and monocyte adhesion to endothelial cells and transmigration through the vessel wall. The leukocytes accumulate in the tissues, forming an inflammatory infiltrate. The actions of TNF on endothelium and leukocytes are critical for local inflammatory responses to microbes. If inadequate quantities of TNF are present (e.g., in patients treated with drugs that block TNF or in TNF gene knockout mice), a consequence may be failure to contain infections.

In addition, TNF, IL-1, and IL-6 produced at inflammatory sites may enter the blood and be delivered to the bone marrow, where they work in concert with colony-stimulating factors to enhance production of neutrophils from bone marrow progenitors and release of mature neutrophils into the blood. In this way, these cytokines increase the supply of cells that can be recruited to the sites of infection and replace leukocytes that are consumed during inflammatory reactions (see Fig. 4.16).

Typically, the leukocyte that is recruited first into sites of inflammation is the neutrophil because it is the most abundant leukocyte in the blood and the most rapid responder to chemotactic signals. Blood monocytes, which become macrophages in the tissue, become increasingly prominent over time and may be the dominant population in some reactions. Neutrophils and monocyte-derived macrophages phagocytose and kill microbes, discussed later, and these functions are enhanced by the opsonization of microbes by complement proteins, antibodies, and other soluble mediators of innate immunity. Furthermore, the recruited phagocytes, especially the macrophages, respond to the microbes and injured cells by secreting more inflammatory cytokines and chemokines, which promotes more leukocyte recruitment and amplifies the acute inflammatory response.

Acute inflammation can develop in minutes to hours and last for days. Chronic inflammation is a process that takes over from acute inflammation if the infection is not eliminated or the tissue injury is prolonged. It usually involves recruitment and activation of monocytes and lymphocytes. Chronic inflammatory sites also often undergo tissue remodeling, with angiogenesis and fibrosis. Although innate immune stimuli contribute to chronic inflammation, the adaptive immune system is usually also involved and cytokines produced by T cells are powerful inducers of chronic inflammation (see Chapter 10).

Ingestion and Killing of Microbes by Activated Phagocytes

Neutrophils and macrophages that are recruited into sites of infections ingest microbes into vesicles by the process of phagocytosis and destroy these microbes (Fig. 4.17)**.** Phagocytosis is an active, energy-dependent process of engulfment of large particles (greater than 0.5 μm in diameter) into vesicles. Phagocytic vesicles fuse with lysosomes, and the ingested particles are destroyed. In this way, the mechanisms of killing, which could potentially injure the phagocyte, are isolated from the rest of the cell.

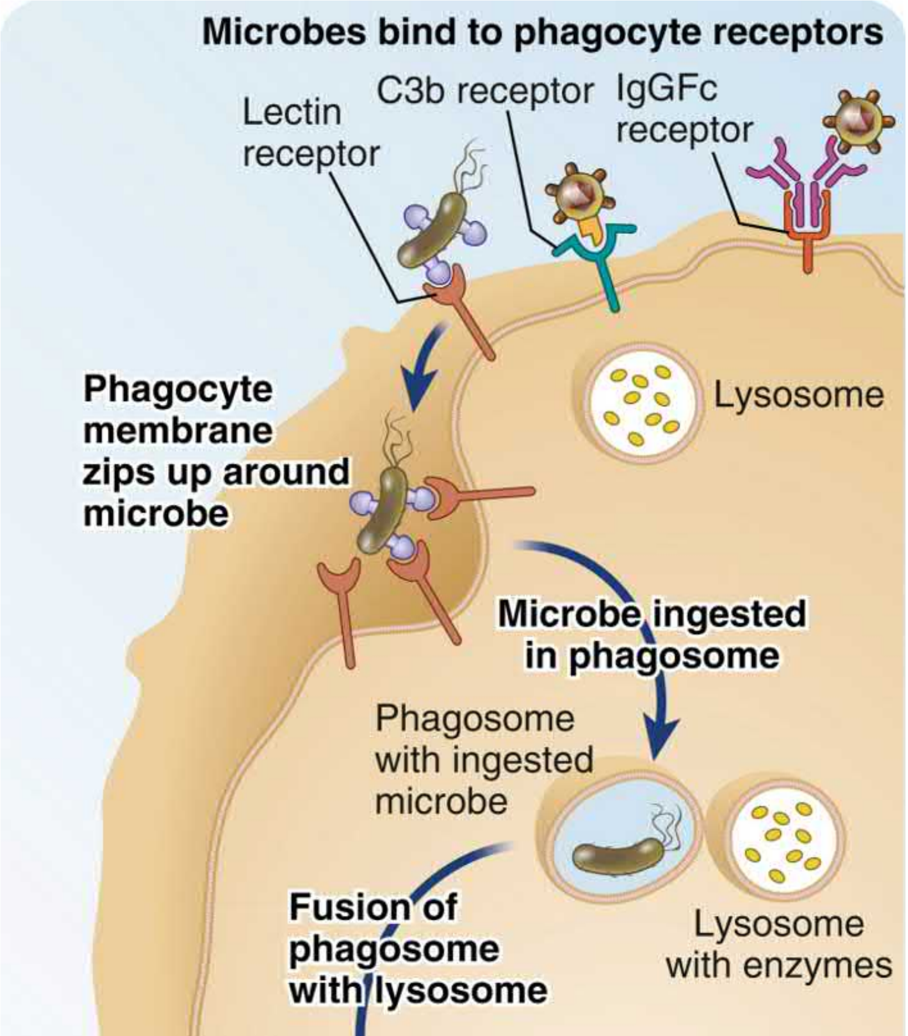

Microbes bind to phagocyte receptors
Lectin receptor
C3b receptor
IgGFc receptor
Lysosome
Phagocyte membrane zips up around microbe
Microbe ingested in phagosome
Phagosome with ingested microbe
Lysosome with enzymes
Fusion of phagosome with lysosome
Activation of phagocyte
Arginine
iNOS
Citrulline
Phago-lysosome
NO
O2
ROS
Phagocyte oxidase
Killing of microbes by ROS, NO, and lysosomal enzymes in phagolysosomes

FIGURE 4.17 Phagocytosis and intracellular destruction of microbes.Microbes may be ingested by different membrane receptors of phagocytes; some directly bind microbes, and others bind opsonized microbes. The microbes are internalized into phagosomes, which fuse with lysosomes to form phagolysosomes, where the microbes are killed by reactive oxygen and nitrogen species and proteolytic enzymes. *IgG,* Immunoglobulin G; *iNOS,* inducible nitric oxide synthase; *NO,* nitric oxide; *ROS,* reactive oxygen species.

Neutrophils and macrophages express receptors that specifically recognize microbes, and binding of microbes to these receptors is the first step in phagocytosis. Some of these receptors are pattern recognition receptors, including C-type lectins and scavenger receptors, which we discussed earlier. Pattern recognition receptors can contribute to phagocytosis only of organisms that express particular molecular patterns, such as mannose for the mannose receptor. Phagocytes also have high-affinity receptors for certain opsonins, including antibody molecules, complement proteins, and plasma lectins; these receptors are critical for phagocytosis of many different microbes that are coated with the opsonins. Coating microbes with antibodies is one of the most efficient systems for opsonization. Phagocytes express a high-affinity Fc receptor called FcγRI, specific for one type of antibody called IgG (see Chapters 5 and 13). Thus, if an individual responds to an infection by making IgG antibodies against microbial antigens, the IgG molecules bind to these antigens, the Fc ends of the bound antibodies can interact with FcγRI on phagocytes, and the end result is efficient phagocytosis of the microbes. Antibody-dependent phagocytosis illustrates a link between innate and adaptive immunity—antibodies are a product of the adaptive immune system (B lymphocytes) that engages innate immune system effector cells (phagocytes) to perform their protective functions.

Once a microbe or particle binds to receptors on a phagocyte, the plasma membrane in the region of the receptors begins to invaginate and extends a cup-shaped projection around the microbe. When the protruding membrane cup extends beyond the diameter of the particle, the top of the cup closes over and pinches off the interior of the cup to form an inside-out intracellular vesicle (see Fig. 4.17). This vesicle, called a phagosome, contains the ingested foreign particle, and it breaks away from the plasma membrane. The cell surface receptors also deliver activating signals that stimulate the microbicidal activities of phagocytes. Phagocytosed microbes are destroyed, as described next. At the same time, peptides are generated from microbial proteins and presented to T lymphocytes to initiate adaptive immune responses (see Chapter 6).

Activated neutrophils and macrophages kill phagocytosed microbes by the action of microbicidal molecules in phagolysosomes (see Fig. 4.17) **.** Signals from various receptors, including pattern recognition receptors (such as TLRs), opsonin receptors (such as Fc and C3b receptors), receptors for cytokines (mainly IFN-γ), and CD40, function cooperatively to activate phagocytes to kill ingested microbes. Fusion of phagocytic vacuoles (phagosomes) with lysosomes results in the formation of phagolysosomes, where most of the microbicidal mechanisms are concentrated. Three classes of microbicidal molecules are known to be the most important.

- ***Reactive oxygen species (ROS).*** Activated neutrophils and, to a lesser extent, macrophages convert molecular oxygen into ROS, which are highly reactive oxidizing agents with free radicals that destroy microbes (and other cells). The primary free radical–generating system is the phagocyte oxidase system. Phagocyte oxidase is a multisubunit enzyme that is assembled in activated phagocytes, mainly in the phagolysosomal membrane. Phagocyte oxidase is activated by many stimuli, including IFN-γ and signals from TLRs. The function of this enzyme is to reduce molecular oxygen into ROS such as superoxide radicals, with the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) acting as a cofactor. Superoxide is enzymatically dismutated into hydrogen peroxide, which is used by the enzyme myeloperoxidase to convert normally unreactive halide ions into reactive hypohalous acids that are toxic for bacteria. The process by which ROS are produced is called the **respiratory burst,** because it requires oxygen consumption (cellular respiration). A disease called **chronic granulomatous disease** is caused by an inherited deficiency of one of the components of phagocyte oxidase; this deficiency compromises the capacity of phagocytes to kill certain species of bacteria (see Chapter 21).

- ***Nitric oxide (NO).*** Macrophages produce reactive nitrogen species, mainly NO, by the action of an enzyme called inducible nitric oxide synthase (iNOS). iNOS is a cytosolic enzyme that is absent in resting macrophages but can be induced in response to microbial products that activate TLRs, especially in combination with IFN-γ. iNOS catalyzes the conversion of arginine to citrulline, and freely diffusible NO gas is released. Within phagolysosomes, NO may combine with hydrogen peroxide or superoxide, generated by phagocyte oxidase, to produce highly reactive peroxynitrite radicals that can kill microbes. The cooperative and redundant function of ROS and NO is demonstrated by the finding that knockout mice lacking both iNOS and phagocyte oxidase are more susceptible to bacterial infections than single phagocyte oxidase or iNOS knockout animals.
- ***Proteolytic enzymes.*** Activated neutrophils and macrophages produce several proteolytic enzymes in the phagolysosomes that function to destroy microbes. One of the important enzymes in neutrophils is elastase, a broad-spectrum serine protease known to be required for killing

many types of bacteria. Another important enzyme is cathepsin G. Mouse gene knockout studies have confirmed the essential requirement for these enzymes in phagocyte killing of bacteria.

Microbes within macrophages may also be killed when the macrophages undergo inflammasome-mediated pyroptosis, as described earlier.

Neutrophils also kill microbes by extruding their DNA and granule contents, which form extracellular threads on which bacteria and fungi are trapped and killed. The extruded chromatin contents, which are called **neutrophil extracellular traps** (NETs), are composed of strands of DNA and histones to which high concentrations of antimicrobial granule contents are bound, including lysozyme, elastase, and defensins. NET formation requires citrullination of histones by the enzyme peptidylarginine deiminase (PAD4), as well as neutrophil serine protease elastase, myeloperoxidase, and phagocyte oxidase. The extrusion of nuclear contents during NET formation leads to neutrophil cell death, referred to as NETosis. The importance of NETs in innate protection against infections remains unclear, but growing evidence indicates that excessive NET formation contributes to autoimmune and other inflammatory diseases.

Role of Macrophages in Tissue Repair

Acute inflammation is often associated with significant death of host cells, including tissue cells killed by microbes or from collateral damage by microbicidal activities of recruited leukocytes. Once the offending agents are eliminated, the damaged tissue has to be repaired. Macrophages play a critical role in the repair process because of several activities. Macrophages clear dead cells and secrete growth factors that promote regeneration and angiogenesis. Macrophages also secrete TGF-β and other cytokines that stimulate collagen synthesis by fibroblasts, thus promoting the formation of scar tissue to replace the damaged parts. Macrophages activated in different ways are responsible for different functions: classically activated macrophages are microbicidal and promote inflammation early in the reaction, and alternatively activated macrophages promote tissue repair later. These pathways of macrophage activation are discussed in more detail in Chapter 10.

Systemic and Pathologic Consequences of Inflammation

TNF, IL-1, and IL-6 produced during the innate immune response to infection or tissue damage have systemic effects that contribute to host defense and are responsible for many of the clinical manifestations of infection and inflammatory disease (see Fig. 4.16) **.**

- **Fever.** TNF and IL-1 act on the hypothalamus to induce an increase in body temperature (fever). These cytokines are therefore called endogenous pyrogens (i.e., host-derived fever-causing agents, to distinguish them from LPS, which was considered an exogenous [microbe-derived] pyrogen). This distinction is mainly of historical significance because we now know that even LPS induces fever by stimulating production of the cytokines TNF and IL-1. These cytokines increase synthesis of prostaglandins in hypothalamic cells, and prostaglandins stimulate the production of neurotransmitters that reset the body's steady-state temperature to a higher level by reducing heat loss (via vasoconstriction) and increasing heat generation (through effects on skeletal muscle and fat). Prostaglandin synthesis inhibitors, such as aspirin, reduce fever by blocking this action of the cytokines. The role of fever in host defense is not well understood but may relate to enhanced metabolic functions of immune cells, impaired metabolic functions of microbes, and changes in the behavior of the febrile host that reduces risk for worsening infections and injury.

- ***Leukocytosis.*** TNF, IL-1, and IL-6 produced at inflammatory sites circulate to the bone marrow and promote the release of neutrophils and monocytes, and other cytokines called colony stimulating factors (see Chapter 2) stimulate production of these cells, leading to elevated numbers of white cells in the blood (leukocytosis). This enhances leukocyte migration into tissues, which can aid in antimicrobial defense but can also contribute to inflammatory damage to tissues.
- ***Acute-phase response.*** TNF, IL-1 and IL-6 induce hepatocytes to produce acute-phase proteins, including CRP, SAP, and fibrinogen, which are secreted into the blood. Elevated plasma levels of these proteins are commonly used clinically as signs of infection or other inflammatory processes. The pentraxins CRP and SAP play protective roles in infections, as we discussed earlier in the chapter, and fibrinogen, the precursor of fibrin, contributes to hemostasis and tissue repair.

In severe infections, TNF may be produced in large amounts and causes systemic clinical and pathologic abnormalities. If the stimulus for cytokine production is sufficiently strong, large amounts of TNF may be made, enter the bloodstream, and act at distant sites (see Fig. 4.16). The principal pathologic actions of TNF are as follows:

- TNF inhibits myocardial contractility and vascular smooth muscle tone, resulting in a marked decrease in blood pressure, or shock.
- TNF causes intravascular thrombosis, mainly as a result of impairment of the normal anticoagulant properties of the endothelium. TNF stimulates endothelial cell expression of tissue factor, a potent activator of coagulation, and inhibits expression of thrombomodulin, an inhibitor of coagulation. The endothelial alterations are exacerbated by activation of neutrophils, leading to vascular plugging by these cells.

- Prolonged production of TNF causes wasting of muscle and fat cells, called cachexia. This wasting results from TNF-induced appetite suppression and reduced synthesis of lipoprotein lipase, an enzyme needed to release fatty acids from circulating lipoproteins so they can be used by the tissues.

A systemic complication of severe infection, usually bacterial or fungal, is a syndrome called **sepsis,** clinically characterized by fever, fast heart and respiratory rates, metabolic abnormalities, and mental disturbances. The infection may involve microbes in the blood, but this is not documented in most cases. Bacterial sepsis is most often initiated by LPS (also called endotoxin) released from gram-negative bacteria or lipoteichoic acid released from gram-positive bacteria, which may enter the blood stream. TLR signaling is then induced in cells in many organs by LPS or lipoteichoic acid, leading to the production of TNF and other cytokines, including IL-12, IFN-γ, IL-6, and IL-1. In the most severe form of sepsis, called **septic shock,** there is vascular collapse and disseminated intravascular coagulation, caused by the effects of high doses of TNF discussed earlier. The concentration of serum TNF may be predictive of the outcome of severe sepsis. Septic shock can be reproduced in experimental animals by administration of LPS, lipoteichoic acid, or TNF. Antagonists of TNF can prevent mortality in the experimental models, but clinical trials with anti-TNF antibodies or with soluble TNF receptors have not shown benefit in patients with sepsis. The cause of this therapeutic failure is not known, but it may be because other cytokines elicit the same responses as TNF.

A syndrome similar to septic shock may occur as a complication of noninfectious disorders, such as severe burns, trauma, pancreatitis, and other serious conditions. This has been called the systemic inflammatory response syndrome (SIRS).

Acute inflammation may cause tissue injury because the effector mechanisms that leukocytes use to kill microbes are also toxic to host tissues. The proteolytic enzymes ROS and NO, produced by phagocytes that accumulate at a site of infection, can injure host cells and degrade extracellular matrix if they are generated in large quantities, especially if the microbes resist being killed and continue to stimulate innate immune responses. In fact, at least part of the pathologic process associated with infections is due to the inflammatory responses and not the direct toxic effects of the microbes. Acute inflammation also causes tissue damage in the setting of autoimmune diseases, in which case neutrophils and macrophages accumulate and become activated as a result of stimulation of the adaptive immune system by self antigens (see Chapter 15). As in inflammation induced by infections, TNF, IL-1, IL-6, and IL-12 are key inducers of inflammation in autoimmune diseases. Antagonists against all of these cytokines or their receptors are in clinical use to reduce inflammation in patients with inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis.

The Antiviral Response

The major way by which the innate immune system blocks viral infections is to induce the expression of type I IFNs, whose most important action is to inhibit viral replication. Earlier in the chapter, we discussed how several pattern recognition receptors, including some TLRs, NLRs, RLRs, and CDSs, generate signals that stimulate IFN-α and IFN-β gene expression in many different cell types. These type I IFNs are secreted from the cells and act on other cells to prevent the spread of viral infection. In this section, we will describe the properties of type I IFNs and the antiviral actions of these cytokines (Fig. 4.18). The antiviral functions of type I interferons are mimicked by type III interferons, made up of four members of the IFN-λ family, which are the main antiviral interferons made by many epithelia and by conventional dendritic cells.

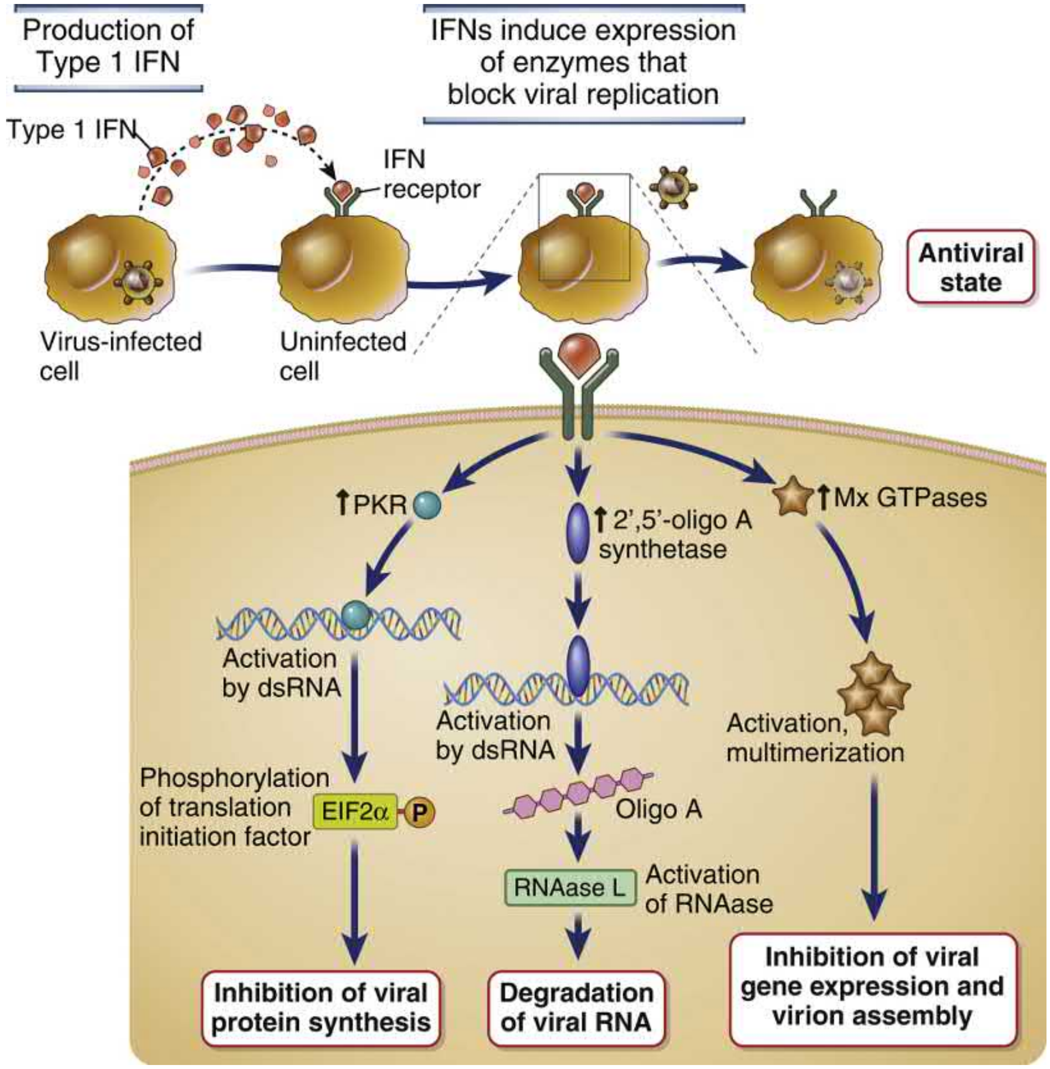

Production of Type 1 IFN
IFNs induce expression of enzymes that block viral replication
Type 1 IFN
IFN receptor
Virus-infected cell
Uninfected cell
Antiviral state
↑PKR
↑2',5'-oligo A synthetase
↑Mx GTPases
Activation by dsRNA
Activation by dsRNA
Activation, multimerization
Phosphorylation of translation initiation factor
EIF2α
P
Oligo A
RNAase L
Activation of RNAase
Inhibition of viral protein synthesis
Degradation of viral RNA
Inhibition of viral gene expression and virion assembly

FIGURE 4.18 Biologic actions of type I interferons. Type I interferons (IFN-α, IFN-β) are produced by virus-infected cells in response to intracellular Toll-like receptor (TLR) signaling and other sensors of viral RNA. Type I IFNs bind to receptors on neighboring uninfected cells and activate JAK-STAT signaling pathways, which induce expression of genes whose products interfere with viral replication. Type I IFNs also bind to receptors on infected cells and induce expression of genes whose products enhance the cell's susceptibility to cytotoxic T lymphocyte (CTL)-mediated killing. *dsRNA,* Double-stranded

RNA; *PKR*, double-stranded RNA-activated protein kinase.

Type I IFNs are a large family of structurally related cytokines that mediate the early innate immune response to viral infections. The term interferon derives from the ability of these cytokines to interfere with viral replication. There are many type I IFNs, which are structurally homologous and are encoded by genes in a single cluster on chromosome 9. The most important type I IFNs in viral defense are IFN-α, which includes 13 closely related proteins, and IFN-β, which is a single protein. Plasmacytoid DCs are the major sources of IFN-α, but it also may be produced by mononuclear phagocytes. IFN-β is produced by many cell types in response to viral infection. The most potent stimuli for type I IFN synthesis are viral nucleic acids. Recall that RIG-like receptors and DNA sensors in the cytosol and TLRs 3, 7, 8, and 9 in endosomal vesicles recognize microbial nucleic acids and initiate signaling pathways that activate the IRF family of transcription factors, which stimulate the transcription of type I IFN genes (see Fig. 4.3).

The receptor for type I IFNs, which binds both IFN-α and IFN-β, is a heterodimer of two structurally related polypeptides, IFNAR1 and IFNAR2, which are expressed on all nucleated cells. This receptor signals to activate STAT1, STAT2, and IRF9 transcription factors, which induce expression of several different genes whose protein products contribute to antiviral defense in various ways:

- Type I IFNs activate transcription of several genes that confer on cells a resistance to viral infection called an antiviral state (see Fig. 4.18). Type I IFN–induced genes include double-stranded RNA–activated serine/threonine protein kinase (PKR), which blocks viral transcriptional and translational events, and 2,5-oligoadenylate synthetase and RNase L, which promote viral RNA degradation. The antiviral action of type I IFN is primarily a paracrine action in that a virally infected cell or a plasmacytoid DC activated by viral PAMPs secretes IFN to act on and protect neighboring cells that are not yet infected. The effects of type I INFs are not specific to viral gene expression, and part of the ability of these cytokines to block the spread of infection is due to their toxicity to host cells that are near infected cells. INF secreted by an infected cell may also act in an autocrine fashion to inhibit viral replication in that cell.

- Type I IFNs cause sequestration of lymphocytes in lymph nodes, thus maximizing the opportunity for encounter with microbial antigens. The mechanism for this effect of type I IFNs is the induction of a molecule on the lymphocytes called CD69, which forms a complex with and reduces surface expression of the sphingosine 1-phosphate (S1P) receptor S1PR1. Recall from Chapter 3 that lymphocyte egress from lymphoid tissues depends on S1P binding to S1PR1. Therefore, reduced S1PR1 inhibits this egress and keeps lymphocytes in lymphoid organs.
- Type I IFNs increase the cytotoxicity of NK cells and $CD8^+$ CTLs and promote the differentiation of naive T cells to the Th1 subset of helper T cells. These effects of type I IFNs enhance both innate and adaptive immunity against intracellular infections, including viruses and some bacteria.
- Type I IFNs upregulate expression of class I MHC molecules and thereby increase the probability that virally infected cells will be recognized and killed by $CD8^+$ CTLs. Virus-specific $CD8^+$ CTLs recognize peptides derived from viral proteins bound to class I MHC molecules on the surface of infected cells. (We will discuss the details of T cell recognition of peptide-MHC and CTL killing of cells in Chapters 6 and 11.) Therefore, by increasing the amount of class I MHC synthesized by a virally infected cell, type I IFNs will increase the number of viral peptide–class I MHC complexes on the cell surface that the CTLs can see and

respond to. The end result is increased killing of virus-infected cells and eradication of viral infections.

Thus, the principal activities of type I IFN work in concert to combat viral infections. Patients who develop severe COVID-19 disease caused by the SARS-CoV-2 virus often show defects in type I IFN. About 10% of severely ill patients produce autoantibodies against their own type I IFN (which may predate the infection), and another 3.5% to 4% have inherited mutations that affect type I IFN production or signaling. Knockout mice lacking the receptor for type I IFNs are susceptible to viral infections. IFN-α is in clinical use as an antiviral agent in certain forms of viral hepatitis. IFN-α is also used for the treatment of some tumors, perhaps because it boosts CTL activity or inhibits cell proliferation. IFN-β is used as a therapy for multiple sclerosis, but the mechanism of its beneficial effect in this disease is not known.

Protection against viruses is due in part to the activation of intrinsic apoptotic death pathways in infected cells and enhanced sensitivity to extrinsic inducers of apoptosis. Viral proteins synthesized in infected cells may be misfolded, and their accumulation triggers an unfolded protein response that may culminate in apoptosis of the infected cells if the misfolded protein accumulation cannot be corrected. In addition, virally infected cells are hypersensitive to TNF-induced apoptosis. Abundant TNF is made by plasmacytoid DCs and macrophages in response to viral infections, in addition to type I IFNs. The type I TNF receptor engages both proinflammatory and proapoptosis pathways, and viral infection can shift this balance toward apoptosis.

Stimulation of Adaptive Immunity

The innate immune response provides signals that function in concert with antigen to stimulate the proliferation and differentiation of antigen-specific T and B lymphocytes. Because the innate immune response is providing the initial defense against microbes, it also sets in motion the adaptive immune response. The activation of lymphocytes requires two distinct signals, the first being antigen and the second being molecules that are produced during innate immune responses to microbes or injured cells (Fig. 4.19). This idea is called the **two-signal hypothesis** for lymphocyte activation. The requirement for antigen (so-called signal 1) ensures that the ensuing immune response is specific. The requirement for additional stimuli triggered by innate immune reactions to microbes (signal 2) ensures that adaptive immune responses are induced when there is a dangerous infection and not when lymphocytes recognize harmless antigens, including self antigens. The molecules produced during innate immune reactions that function as second signals for lymphocyte activation include costimulators (for T cells), cytokines (for both T and B cells), and complement breakdown products (for B cells). We will return to the nature of second signals for lymphocyte activation in Chapters 9 and 12.

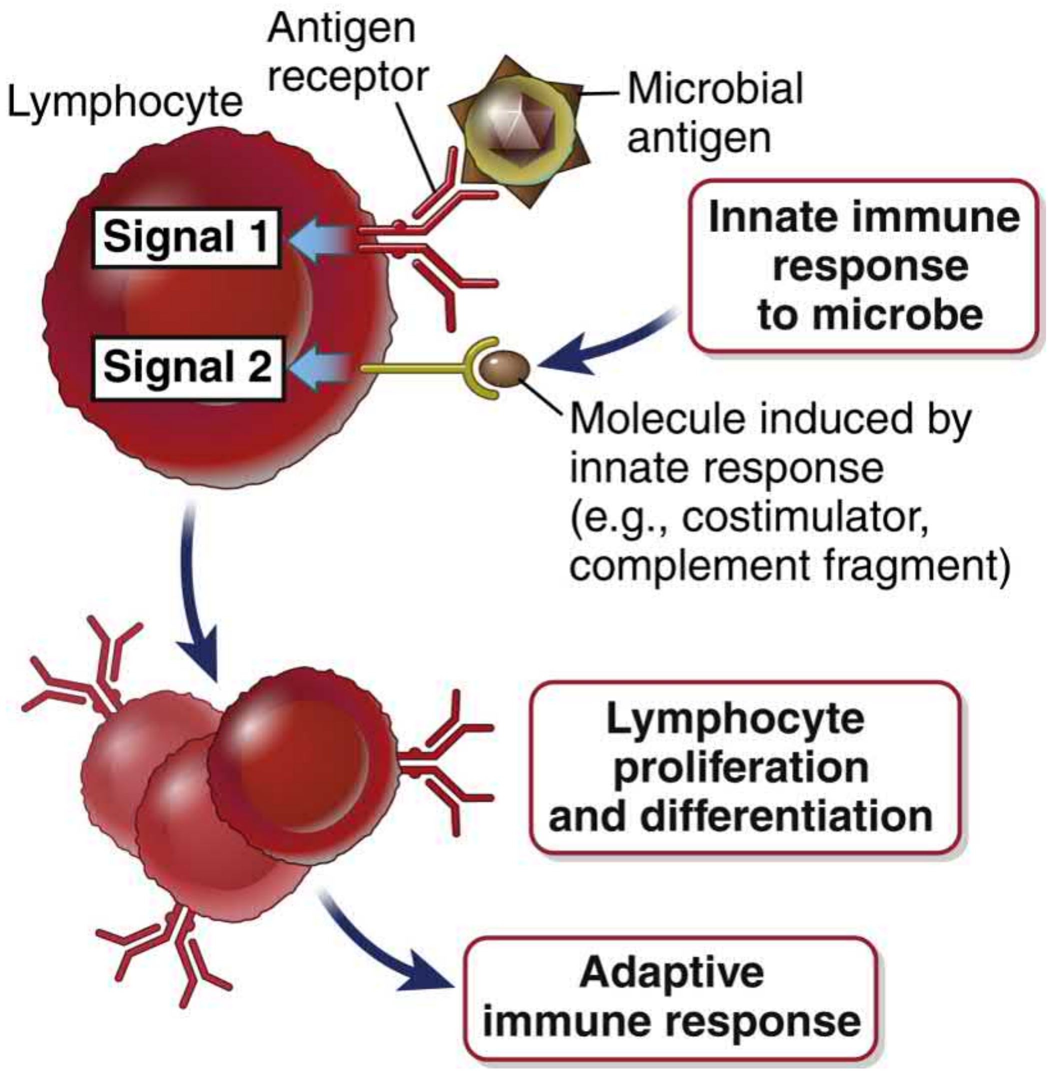

Lymphocyte
Antigen receptor
Microbial antigen
Signal 1
Signal 2
Innate immune response to microbe
Molecule induced by innate response (e.g., costimulator, complement fragment)
Lymphocyte proliferation and differentiation
Adaptive immune response

FIGURE 4.19 Stimulation of adaptive immunity by innate immune responses.Antigen recognition by lymphocytes provides signal 1 for the activation of the lymphocytes, and molecules induced on host cells during innate immune responses to microbes provide signal 2. In this illustration, the lymphocytes are B cells, but the same principles apply to T lymphocytes. The nature of second signals differs for B and T cells and is described in later chapters.

The second signals generated during innate immune responses to different microbes not only enhance the magnitude of the subsequent

adaptive immune response but also influence the nature of the adaptive response. A major function of T cell–mediated immunity is to activate macrophages to kill intracellular microbes and to induce robust inflammatory responses so that a sufficiently large army of phagocytes is called into a site of infection. When DCs or phagocytes encounter microbes, TLRs and other pattern recognition receptors stimulate the secretion of cytokines and induce on antigen-presenting cells the expression of molecules, called costimulators, all of which promote T cell activation (see Chapter 9). Thus, the innate immune response to microbes in macrophages stimulates the adaptive T cell response that helps the macrophages to destroy such microbes.

By contrast, many extracellular microbes that enter the blood activate the alternative complement pathway, and some of the proteolytic products of complement activation enhance the production of antibodies by B lymphocytes (see Chapter 12). These antibodies opsonize the microbes and thereby promote their phagocytosis by neutrophils and macrophages, or kill the microbes by complement-dependent mechanisms. Thus, blood-borne microbes induce an innate response (complement activation) that triggers the adaptive response that is designed to eliminate these extracellular pathogens.

Cytokines produced by cells during innate immune responses to microbes or cell injury stimulate the proliferation and differentiation of lymphocytes in adaptive immune responses. Examples of cytokines secreted during innate immune reactions acting on B cells, CD4$^+$ T cells, and CD8$^+$ T cells are given here. We have mentioned these cytokines previously and will discuss the details of their roles in lymphocyte responses in later chapters.

- IL-12 stimulates the differentiation of naive CD4$^+$ T cells to the Th1 subset of effector cells (see Chapter 10) and naive CD8$^+$ T cells to CTLs.
- IL-1, IL-6, and IL-23 stimulate the differentiation of naive CD4$^+$ T cells to the Th17 subset of effector cells (see Chapter

10).

- IL-25, IL-33, and TSLP stimulate the differentiation of naive $CD4^+$ T cells to the Th2 subset of effector cells.
- IL-15 promotes the survival of memory $CD8^+$ T cells.
- IL-6 promotes the survival of antibody-producing plasma cells.

Adjuvants are substances that need to be administered together with purified protein antigens to elicit maximal T cell–dependent immune responses (see Chapter 6). They work by stimulating innate immune responses at the site of antigen exposure that promote subsequent adaptive immunity. Adjuvants are useful in experimental immunology and in clinical vaccines. Many adjuvants in experimental use are microbial products that engage TLRs, such as killed mycobacteria and LPS. The most commonly used adjuvant in human vaccines is alum, which is composed of either aluminum hydroxide or aluminum phosphate, and may work by causing inflammasome activation. CpG, the ligand for TLR9, is used in some hepatitis B virus vaccines. Among their important effects, adjuvants activate DCs to express more major histocompatibility molecules that are part of the antigen (signal 1) that T cells recognize, increase the expression of costimulators (signal 2) and cytokines needed for T cell activation, and stimulate migration of the DCs to lymph nodes where T cells are located.

Mechanisms that Limit Innate Immune Responses

The magnitude and duration of innate immune responses are regulated by a variety of inhibitory mechanisms that limit potential damage to tissues. Whereas the inflammatory response is critically important for protection against microbes, it has the potential to cause tissue injury and disease. Several mechanisms have evolved to provide a brake on inflammation, and these mechanisms come into play at the same time as or shortly after the initiation of inflammation. Furthermore, the stimuli for the initiation of many of these control mechanisms include the same PAMPs and DAMPs that induce inflammation. We will describe a selected group of these regulatory mechanisms.

IL-10 is a cytokine that is produced by and inhibits activation of macrophages and DCs. IL-10 inhibits the production of various inflammatory cytokines by activated macrophages and DCs, including IL-1, TNF, and IL-12. Because it is both produced by macrophages and DCs and inhibits the functions of these cells, IL-10 is an example of a negative feedback regulator. Alternatively activated macrophages make more IL-10 than classically activated macrophages. IL-10 is produced by some nonhematopoietic cell types (e.g., keratinocytes). IL-10 is also produced by regulatory T cells, and we will discuss the details of IL-10 in this context in Chapter 15. Loss-of-function mutations in the IL-10 receptor result in severe colitis developing in infancy.

Macrophages produce a natural antagonist of IL-1 that is structurally homologous to the cytokine and binds to the same receptors but is biologically inactive, so that it functions as a competitive inhibitor of IL-1. It is therefore called **IL-1 receptor antagonist (IL-1RA)**. Synthesis of IL-1RA is induced by many of the same stimuli that induce IL-1 production. Some studies in IL-1RA–deficient mice suggest that this inhibitory cytokine is required to prevent inflammatory diseases of joints and other tissues, and a rare disease caused by genetic deficiency of IL-1RA in humans is characterized by severe bone and skin inflammation. Recombinant IL-1RA has been developed as a drug for the treatment of rheumatoid arthritis and familial fever syndromes in which IL-1 production is dysregulated. Regulation of IL-1–mediated inflammation may also occur by expression of the type II receptor, which binds IL-1 but does not transduce an activating signal. The major function of this receptor may be to act as a decoy that competitively inhibits IL-1 binding to the type I signaling receptor.

There are numerous negative regulatory signaling pathways that block the activating signals generated by pattern recognition receptors and inflammatory cytokines. Suppressors of cytokine signaling (SOCS) proteins are inhibitors of JAK-STAT signaling pathways linked to cytokine receptors. TLR signaling in macrophages and DCs induces the expression of SOCS proteins, which limit responses of these cells to exogenous cytokines such as type I IFNs. Proinflammatory responses of cells to TLR signaling are negatively regulated by SHP1, an intracellular protein phosphatase that negatively regulates numerous tyrosine kinase–dependent signaling pathways in lymphocytes. There are many other examples of kinases and phosphatases that inhibit TLR, NLR, and RLR signaling and small inhibitory RNAs that inhibit production of many of the mediators of innate immunity.

Summary

- The innate immune system provides the first line of host defense against microbes, before adaptive immune responses have had sufficient time to develop. The mechanisms of innate immunity exist before exposure to microbes. The cellular components of the innate immune system include epithelial barriers and leukocytes (neutrophils, macrophages, natural killer [NK] cells, lymphocytes with invariant antigen receptors, and mast cells).
- The innate immune system uses cell-associated pattern recognition receptors, present on plasma and endosomal membranes and in the cytosol, to recognize structures called pathogen-associated molecular patterns (PAMPs), which are shared by microbes, are not present on mammalian cells, and are often essential for survival of the microbes, thus limiting the capacity of microbes to evade detection by mutating or losing expression of these molecules. In addition, these receptors recognize molecules made by the host but whose expression or location indicates cellular damage; these are called damage-associated molecular patterns (DAMPs).

- Toll-like receptors (TLRs), present on the cell surface and in endosomes, are an important family of pattern recognition receptors, recognizing a wide variety of ligands, including bacterial cell wall components and microbial nucleic acids. Cytosolic pattern recognition receptors exist that recognize microbial molecules. These receptors include the retinoic acid–inducible gene (RIG)-like receptors (RLRs), which recognize viral RNA; cytosolic DNA sensors (CDSs), which recognize microbial DNA; and NOD-like receptors (NLRs), which recognize bacterial cell wall constituents and also serve as recognition components of many inflammasomes.
- Pattern recognition receptors, including TLRs, NLRs, and RLRs, signal to activate the transcription factor NF-κB, which stimulates expression of cytokines, costimulators, and other molecules involved in inflammation, and the interferon response factor (IRF) transcription factors, which stimulate expression of the antiviral type I interferon (IFN) genes.
- The inflammasome, a specialized caspase-1–containing enzyme complex that forms in response to a wide variety of PAMPs and DAMPs, includes recognition structures, which are often NLR family proteins, an adaptor, and the enzyme caspase-1, the main function of which is to produce active forms of the inflammatory cytokines interleukin 1 (IL-1) and IL-18.
- Inflammasome-mediated proteolytic processing of the cytosolic protein gasdermin generates membrane pores, which are a conduit for release of IL-1 from the cell and also cause osmotic cell death, called pyroptosis.
- Soluble effector molecules of innate immunity are found in the plasma and include pentraxins (e.g., C-reactive protein [CRP]), collectins (e.g., mannose-binding lectin [MBL]), and ficolins. These molecules bind microbial ligands and enhance clearance by complement-dependent and complement-independent mechanisms.

- Innate lymphoid cells are cells with lymphocyte morphology and functions similar to those of T lymphocytes, but they do not express clonally distributed T cell antigen receptors. Three helper subsets of ILCs secrete the same cytokines as Th1, Th2, and Th17 helper T cells.
- NK cells have cytotoxic functions and secrete interferon-γ (IFN-γ), similar to cytotoxic T lymphocytes (CTLs). NK cells defend against intracellular microbes by killing infected cells and providing a source of the macrophage-activating cytokine IFN-γ. NK cell recognition of infected cells is regulated by a combination of activating and inhibitory receptors. Inhibitory receptors recognize class I major histocompatibility complex (MHC) molecules, because of which NK cells do not kill normal host cells but do kill cells in which class I MHC expression is reduced, such as virus-infected cells.
- The complement system includes several plasma proteins that become activated in sequence by proteolytic cleavage to generate fragments of the C3 and C5 proteins, which promote inflammation, or opsonize and promote phagocytosis of microbes. Complement activation also generates membrane pores that kill some types of bacteria. The complement system is activated on microbial surfaces and not on normal host cells, because microbes lack regulatory proteins that inhibit complement. In innate immune responses, complement is activated mainly spontaneously on microbial cell surfaces and by MBL to initiate the alternative and lectin pathways, respectively.
- The two major effector functions of innate immunity are to induce inflammation, which involves the delivery of microbe-killing leukocytes and soluble effector molecules from blood into tissues, and to block viral infection of cells mainly by the antiviral actions of type I IFNs. Both types of effector mechanisms are induced by PAMPs and DAMPs.
- Several cytokines produced mainly by macrophages, dendritic cells (DCs), and other innate immune cells

mediate inflammation. Tumor necrosis factor (TNF) and IL-1 activate endothelial cells, stimulate chemokine production, and increase neutrophil production in the bone marrow. IL-1 and TNF both induce IL-6 production, and all three cytokines mediate systemic effects, including fever and acute-phase protein synthesis by the liver. IL-12 and IL-18 stimulate production of the macrophage-activating cytokine IFN-γ by NK cells and T cells. These cytokines function in innate immune responses to different classes of microbes, and some (IL-1, IL-6, IL-12, IL-18) modify adaptive immune responses that follow the innate immune response.

- Neutrophils and monocytes (the precursors of tissue macrophages) migrate from blood into inflammatory sites during innate immune responses because of the effects of cytokines and chemokines produced by PAMP- and DAMP-stimulated tissue cells.
- Neutrophils and macrophages phagocytose microbes and kill them by producing reactive oxygen species, nitric oxide, and enzymes in phagolysosomes. Macrophages also produce cytokines that stimulate inflammation and promote tissue repair at sites of infection. Phagocytes recognize and respond to microbial products by several different types of receptors, including TLRs, C-type lectins, scavenger receptors, and *N*-formyl met-leu-phe receptors.
- Molecules produced during innate immune responses stimulate adaptive immunity and influence the nature of adaptive immune responses. DCs activated by microbes produce cytokines and costimulators that enhance T cell activation and differentiation into effector T cells. Complement fragments generated by the alternative pathway provide second signals for B cell activation and antibody production.
- Innate immune responses are regulated by negative feedback mechanisms that limit potential damage to tissues. IL-10 is a cytokine that is produced by and inhibits

activation of macrophages and DCs. Inflammatory cytokine secretion is regulated by autophagy gene products. Negative signaling pathways block the activating signals generated by pattern recognition receptors and inflammatory cytokines.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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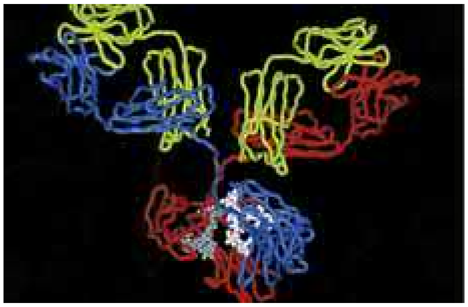
Chapter 5: Antibodies and Antigens



- Antibody Structure,
  - General Features of Antibody Structure,
  - Structural Features of Antibody Variable Regions,
  - Structural Features of Antibody Constant Regions,
  - Monoclonal Antibodies,
- Synthesis, Assembly, and Expression of Immunoglobulin Molecules,
  - Half-Life of Antibodies,
- Antibody Binding of Antigens,
  - Features of Biologic Antigens,
  - Structural and Chemical Basis of Antigen Binding,
- Structure-Function Relationships in Antibody Molecules,
  - Features Related to Antigen Recognition,
  - Features Related to Effector Functions,

Summary,

Antibodies are circulating proteins that are produced in vertebrates in response to exposure to foreign structures known as antigens and are the mediators of humoral immunity against all classes of microbes. Antibodies are extremely diverse and specific in their ability to recognize foreign molecular structures. Because these proteins were discovered as circulating molecules that provided protection against diphtheria toxin, they were initially called antitoxins. When it was appreciated that similar proteins could be generated against many substances, not just microbial toxins, they were given the general name **antibodies.** The substances that stimulated production of or were recognized by antibodies were then called **antigens.** Antibodies and T cell antigen receptors (see Chapter 7) are the two classes of molecules used by the adaptive immune system to specifically recognize and respond to antigens (Table 5.1). Major histocompatibility complex (MHC) molecules also bind peptide antigens, but their specificity is very different and their function is to passively display peptides to T cell antigen receptors, not to respond to antigens in the way that antibodies on B lymphocytes and T cell receptors (TCRs) on T lymphocytes do (see Chapter 6). Antibodies were discovered almost a century before antigen receptors were identified on T cells, and in comparison to TCRs and MHC molecules, antibodies recognize a wider range of antigenic structures, have a greater ability to discriminate among different antigens, and bind antigens with the greatest strength. In this chapter we describe the structure and antigen-binding properties of antibodies.

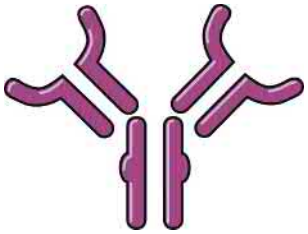
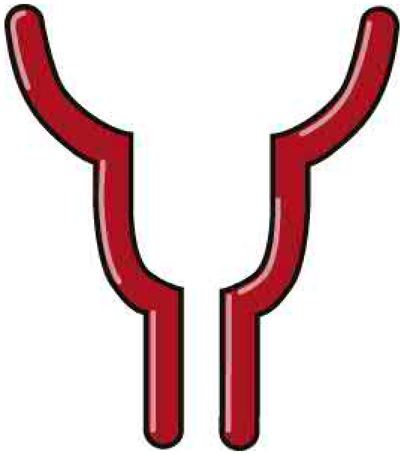
Antibodies are synthesized only by cells of the B lymphocyte lineage and exist in two forms: membrane-bound antibodies on the surface of B lymphocytes function as antigen receptors, and secreted antibodies function to protect against microbes. The recognition of antigens by membrane-bound antibodies on naive B cells activates these lymphocytes and initiates a humoral immune response. The activated B cells differentiate into plasma cells that secrete antibodies of the same specificity as the antigen receptor. Secreted forms of antibodies are present in the plasma (the fluid portion of the blood), in mucosal secretions, and in the interstitial fluid of tissues.

Antibodies recognize antigens and also have effector functions that contribute to the elimination of the antigens. Secreted antibodies neutralize microbial toxins, prevent the entry and spread of pathogens, and trigger several effector mechanisms that eliminate the microbes. As we will discuss later, different regions of antibody molecules are responsible for recognition of antigens and many of the effector functions, but all functions of antibodies are initiated by specific antigen recognition. The elimination of antigens often requires interaction of antibodies with other components of the immune system, including molecules such as complement proteins and cells such as phagocytes and mast cells. Antibody-mediated effector functions include neutralization of microbes or toxic microbial products; activation of the complement system; opsonization of pathogens for enhanced phagocytosis; antibody-dependent cell-mediated cytotoxicity, by which antibodies target infected cells for lysis by cells of the innate immune system; and antibody-mediated mast cell activation to expel parasitic worms. We will describe these functions of antibodies in detail in Chapter 13.

When blood or plasma removed from an individual and placed in a tube forms a clot, antibodies remain in the residual fluid, which is called **serum.** Serum lacks coagulation factors (which are consumed during clot formation) but contains all the other proteins found in plasma. Any serum sample that contains detectable antibody molecules that bind to a particular antigen is commonly called an **antiserum.** The study of antibodies and their reactions with antigens is therefore called **serology.** The concentration of antibody molecules in serum specific for a particular antigen is often estimated by determining how many serial dilutions of the serum can be made before binding to the antigen can no longer be detected. This process of determining the concentration of any substance by testing serial dilutions is called titration, and the antibody concentration determined by this method is the titer. The more dilutions that are required, the higher is the titer of the antibody molecules specific for a particular antigen.

TABLE 5.1

Features of Antigen Binding by Antigen Receptors [a]

Feature	Immunoglobulin (Ig)	T Cell Receptor (TCR)
		
Antigen-binding site	Made up of three CDRs in V_H and three CDRs in V_L domains	Made up of three CDRs in $V\alpha$ and three CDRs in $V\beta$ domains (in the most common form of TCR)
Nature of antigen that may be bound	Macromolecules (proteins, lipids, polysaccharides) and small chemicals	Peptide-MHC complexes
Nature of antigenic determinants recognized	Linear and conformational determinants of various macromolecules and chemicals	Linear determinants of peptides; only few amino acid residues of a peptide bound to an MHC molecule

Feature	Immunoglobulin (Ig)	T Cell Receptor (TCR)
Affinity of antigen binding	K_d 10^{-7}–10^{-11} M; average affinity of Igs increases during immune response	K_d 10^{-5}–10^{-7} M
On-rate and off-rate	Rapid on-rate, variable off-rate	Slow on-rate, slow off-rate

CDR, Complementarity-determining region; K_d, dissociation constant; *MHC,* major histocompatibility complex; V_H, variable domain of heavy chain Ig; V_L, variable domain of light chain Ig; *V*α, *V*β, variable domains of TCR α and β chains.

[a] The structure and function of TCR molecules are discussed in Chapter 7.

A healthy 70-kg adult human produces about 2 to 3 g of antibodies every day. Almost two-thirds of this is a type of antibody called immunoglobulin A (IgA), most of which is produced by intestinal plasma cells and secreted into the gut lumen.

Antibody Structure

An understanding of the structure of antibodies has provided important insights into their function. The analysis of antibody structure also laid the foundation for elucidating the mechanisms of antigen receptor diversity that we will consider in depth in Chapter 8.

Early studies of antibody structure relied on antibodies purified from the blood of individuals immunized with various antigens. It was not possible, using this approach, to define antibody structure precisely because serum contains a mixture of different antibodies produced by many clones of B lymphocytes that may each bind to different portions (epitopes) of an antigen. These antibody mixtures are called polyclonal antibodies. A major breakthrough in obtaining antibodies whose structures could be elucidated was the discovery that patients with multiple myeloma, a monoclonal tumor of antibody-producing plasma cells, often have large amounts of biochemically identical antibody molecules (produced by the neoplastic clone) in their blood and urine. Immunologists found that these antibodies could be purified to homogeneity and analyzed. The realization that myeloma cells make one type of immunoglobulin led to the development of the technology to produce monoclonal antibodies, described later in the chapter. The availability of homogeneous populations of antibodies and monoclonal antibody–producing plasma cells facilitated the detailed structural analysis of antibody molecules and the molecular cloning of the genes for individual antibodies. These were important advances in our understanding of the adaptive immune system.

General Features of Antibody Structure

Plasma or serum proteins can be physically separated based on solubility characteristics into albumins and globulins and may be more precisely separated, based on differences in charge, using a technique called electrophoresis. In electrophoretic separations of serum or plasma, most antibodies are found in the third-fastest migrating group of globulins, named **gamma globulins** for the third letter of the Greek alphabet. (Note that gamma globulins include all classes of antibodies, described later, not just the IgG class.) Another common name for antibody is **immunoglobulin (Ig),** referring to the immunity-conferring portion of the globulin fraction of serum or plasma. The terms immunoglobulin and antibody are used interchangeably throughout this book.

All antibody molecules share the same basic structural characteristics but display remarkable variability in the regions that bind antigens. This variability of the antigen-binding regions accounts for the capacity of different antibodies to bind a tremendous number of structurally diverse antigens. In every individual, there are millions of different clones of B cells, each producing antibody molecules with identical antigen-binding sites but that differ from the antigen-binding sites of antibodies produced by other clones. The effector functions and common physicochemical properties of antibodies are associated with the non–antigen-binding portions, which exhibit relatively few variations among different antibodies.

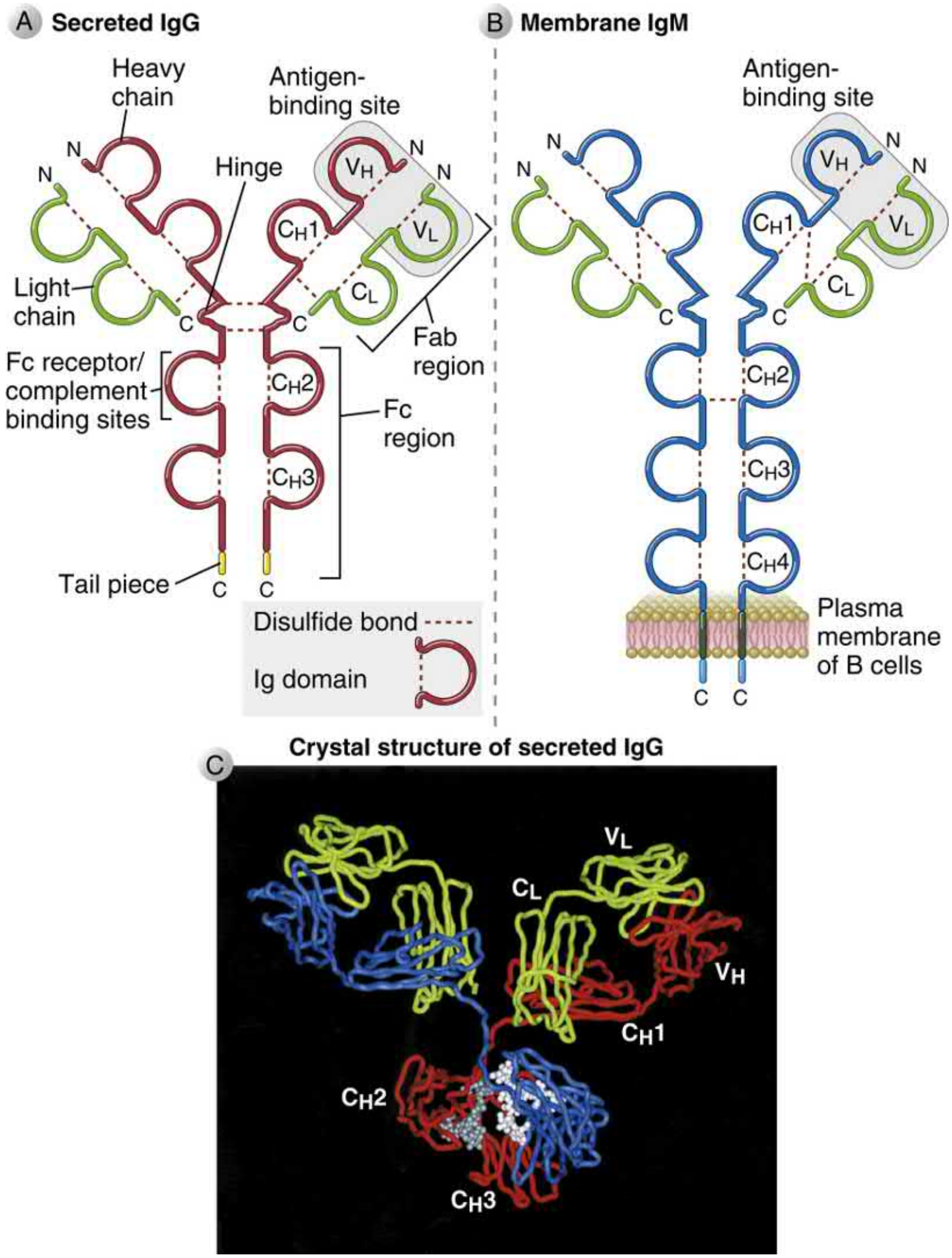

A Secreted IgG
Heavy chain
Antigen-binding site
Hinge
N
N
N
N
VH
VL
CH1
CL
Light chain
C
C
Fab region
Fc receptor/complement binding sites
CH2
CH3
Fc region
Tail piece
C
C
Disulfide bond
Ig domain
B Membrane IgM
Antigen-binding site
N
N
N
N
VH
VL
CH1
CL
C
C
CH2
CH3
CH4
Plasma membrane of B cells
C
C
C Crystal structure of secreted IgG
VL
CL
VH
CH1
CH2
CH3

FIGURE 5.1 Structure of an antibody molecule. **(A)** Schematic diagram of a secreted immunoglobulin G *(IgG)* molecule. The antigen-binding sites are formed by the juxtaposition of V_L and V_H domains. The heavy chain C regions end in tail pieces. The locations of complement-binding and Fc receptor–

binding sites within the heavy chain constant regions are approximations. **B,** Schematic diagram of a membrane-bound IgM molecule on the surface of a B lymphocyte. The IgM molecule has one more C_H domain than IgG has, and the membrane form of the antibody has C-terminal transmembrane and cytoplasmic portions that anchor the molecule in the plasma membrane. **C,** Structure of a human IgG molecule as revealed by x-ray crystallography. In this ribbon diagram of a secreted IgG molecule, the identical heavy chains are colored *blue* and *red* so that they can be easily visualized, although they are identical, and the light chains are colored *green;* carbohydrates that are covalently bound to the Ig heavy chain proteins are shown in *gray.*

Courtesy Dr. Alex McPherson, University of California, Irvine.

An antibody molecule has a symmetric core structure composed of two identical light chains and two identical heavy chains (Fig. 5.1). Both the light chains and heavy chains contain a series of repeating homologous structural units, each about 110 amino acid residues in length, that fold independently in a globular motif that is called an **Ig domain,** which we introduced in Chapters 3 and 4. An Ig domain contains two layers of β-pleated sheet, each layer composed of three to five strands of antiparallel polypeptide chain (Fig. 5.2). The two layers are held together by a disulfide bridge, and adjacent strands of each β sheet are connected by short loops. It is the amino acids in some of these loops that are the most variable and critical for antigen recognition, as discussed later in the chapter.

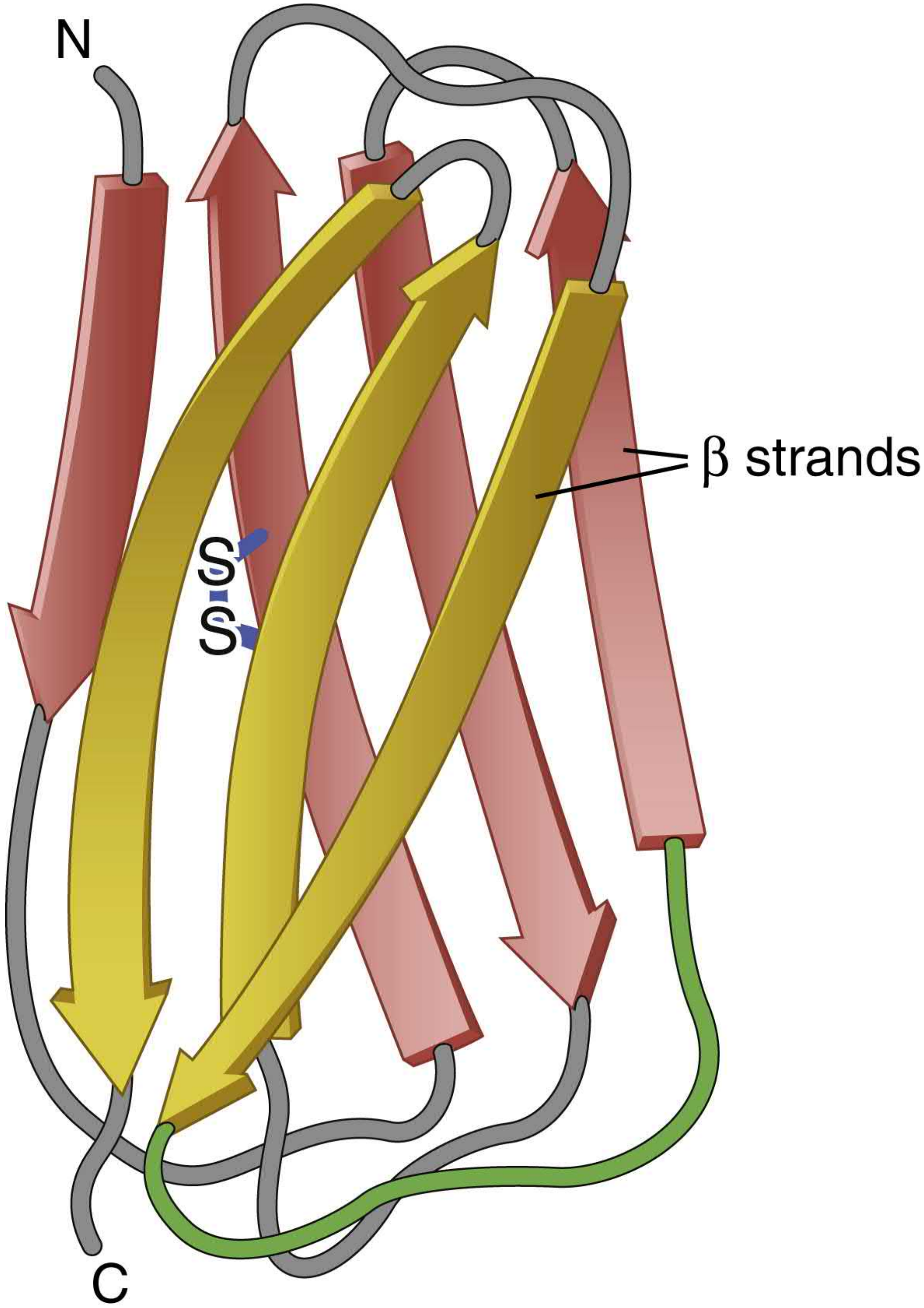


FIGURE 5.2 Structure of an immunoglobulin domain. Each domain is composed of two antiparallel arrays of β strands, colored *yellow* and *red,* to form two β-pleated sheets held

together by a disulfide bond. The diagram shows an immunoglobulin constant *(C)* domain containing three and four β strands in the two adjacent sheets. *N* and *C* at the ends of polypeptide chains refer to the amino and carboxy termini, respectively.

Antibody heavy chains and light chains both consist of amino-terminal variable (V) regions that participate in antigen recognition and carboxy-terminal constant (C) regions; the C regions of the heavy chains help mediate some of the effector functions of antibodies. In the heavy chains, the V region is composed of one Ig domain, and the C region is composed of three or four Ig domains. Each light chain is composed of one V region Ig domain and one C region Ig domain. Variable regions are so named because their amino acid sequences vary among antibodies made by different B cell clones. The V region of one heavy chain (V_H) and the adjacent V region of one light chain (V_L) form an antigen-binding site (see Fig. 5.1). Because the core structural unit of each antibody molecule contains two heavy chains and two light chains, every antibody molecule has at least two antigen-binding sites.

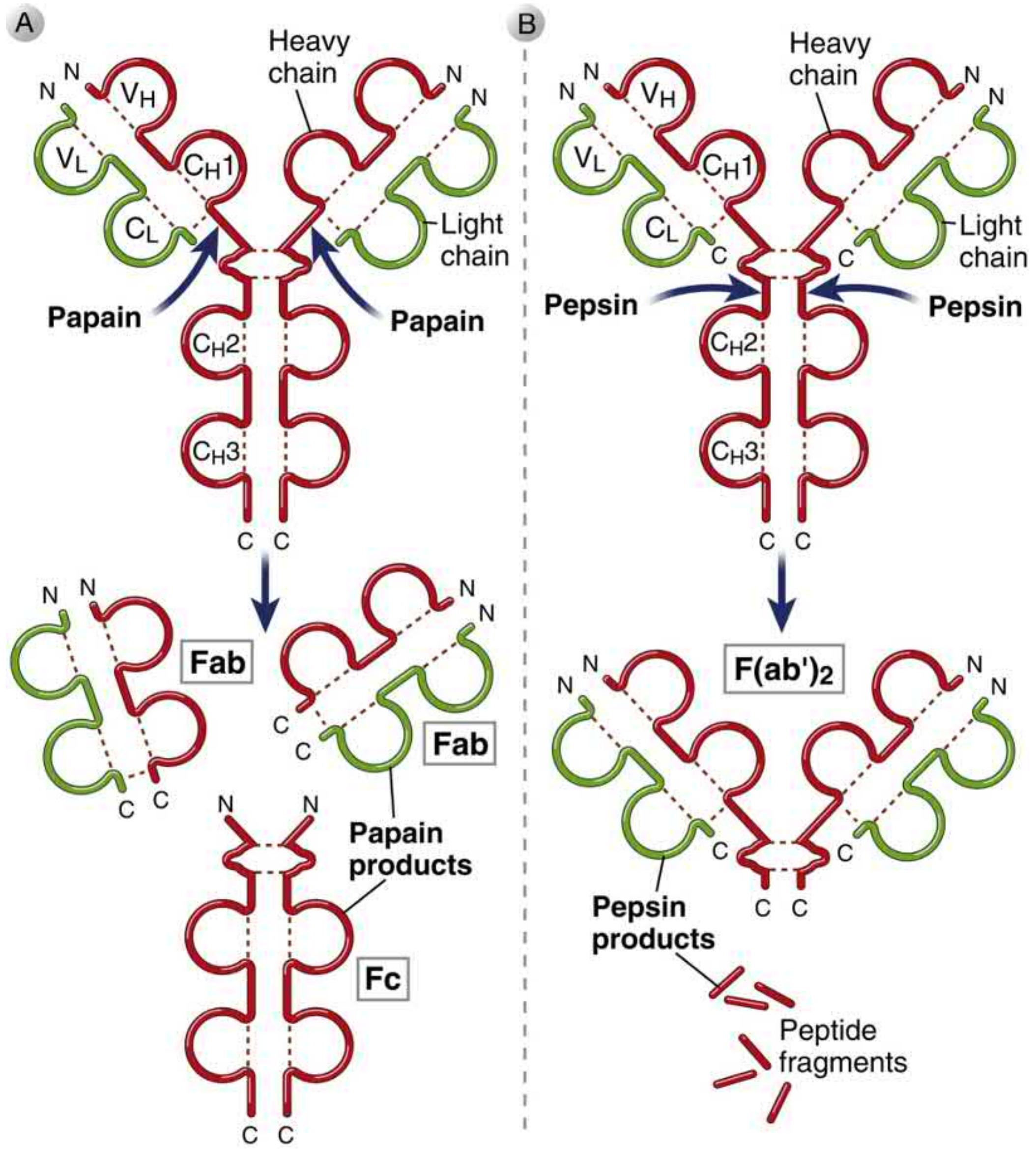
The C region Ig domains are spatially separated from the antigen-binding sites and do not participate in antigen recognition. The heavy chain C regions interact with other molecules and cells of the immune system and therefore help mediate most of the effector functions of antibodies. In addition, heavy chains exist in two forms that differ at their carboxy-terminal ends: one form of the heavy chain anchors membrane-bound antibodies in the plasma membranes of B lymphocytes, and the other form is found only in secreted antibodies. The C regions of light chains do not participate in effector functions and are not directly attached to cell membranes.

Heavy and light chains are covalently linked by disulfide bonds formed between cysteine residues in the carboxy terminus of the light chain and the C_H1 domain of the heavy chain. Noncovalent interactions between the V_L and V_H domains and between the C_L and C_H1 domains may also contribute to the association of heavy and light chains. The two heavy chains of each antibody molecule are also covalently linked by disulfide bonds. There are different kinds of antibodies, called classes or isotypes, that have different heavy chain structures, discussed in detail later in the chapter. In the IgG isotype, these disulfide bonds are formed between cysteine residues in the C_H2 domains, close to an unfolded segment called the **hinge region,** which connects the C_H1 domain to the C_H2 region; this segment is described in more detail later in the chapter. In other isotypes, the disulfide bonds may be in different locations. Noncovalent interactions (e.g., between the third C_H domains [C_H3s]) also contribute to heavy chain pairing.

The antigen-binding portion of an antibody molecule is the Fab region, and the C-terminal end that is involved in effector functions is the Fc region. These regions were originally identified by proteolysis of rabbit IgG molecules. In these molecules, the hinge region between the C_H1 and C_H2 domains of the heavy chain is the segment most susceptible to proteolytic cleavage, because this region is unfolded and proteolytic sites are thus easily accessible. If rabbit IgG is treated with the enzyme papain under conditions of limited proteolysis, the enzyme acts on the hinge region and cleaves the IgG into three separate pieces (Fig. 5.3A). Two of the pieces are identical to each other and consist of the complete light chain (V_L and C_L) associated with a V_H-C_H1 fragment of the heavy chain. These fragments retain the ability to bind antigen because each contains paired V_L and V_H domains, and they are called **Fab** (fragment, antigen binding). The third piece is composed of two identical disulfide-linked peptides, each containing the heavy chain C_H2 and C_H3 domains. This piece of IgG has a propensity to self-associate and to crystallize into a lattice and is therefore called **Fc** (fragment, crystallizable). When pepsin (instead of papain) is used to cleave rabbit IgG under limiting conditions, proteolysis occurs distal to the hinge region, generating a $F(ab')_2$ fragment of IgG with the hinge and the interchain disulfide bonds intact and two identical antigen-binding sites (Fig. 5.3B).

The basic organization of the antibody molecule deduced from the rabbit IgG proteolysis experiments is common to all Ig molecules of all classes and all species, and the terms Fab, $F(ab')_2$, and Fc are widely used to describe these different portions of antibodies. In fact, these experiments provided the first evidence that the antigen recognition functions and the effector functions of Ig molecules are spatially separated.

Many other proteins in the immune system, as well as numerous proteins with no known immunologic function, contain domains with an Ig fold structure—that is, two adjacent β-pleated sheets held together by a disulfide bridge. All molecules that contain this type of domain are said to belong to the **Ig superfamily,** and all gene segments encoding the Ig domains of these molecules are thought to have evolved from one ancestral gene. Ig domains are classified as V-like or C-like on the basis of closest homology to either Ig V or Ig C domains. V domains are formed from a longer polypeptide than C domains and contain two extra β strands within the β sheet sandwich. Some members of the Ig superfamily were described in Chapter 3 (endothelial adhesion molecules ICAM-1 and VCAM-1) and Chapter 4 (NK cell KIR receptors). Examples of Ig superfamily members of relevance in the immune system are depicted in Fig. 5.4.


A
Heavy chain
N
N
VH
VL
CH1
CL
Light chain
Papain
Papain
CH2
CH3
C C
N N
Fab
Fab
C C
C C
N N
Papain products
Fc
C C
B
Heavy chain
N N
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CH1
CL
C
C
Light chain
Pepsin
Pepsin
CH2
CH3
C C
F(ab')2
N N
C C
C C
Pepsin products
Peptide fragments

FIGURE 5.3 Proteolytic fragments of an immunoglobulin G (IgG) molecule.Early studies of the properties of proteolytic fragments of rabbit Ig provided key insights into the general structural features of all Ig molecules. Rabbit IgG molecules are cleaved by the enzymes papain **(A)** and pepsin **(B)** at the sites indicated by *arrows.* Papain digestion allows separation of two antigen-binding regions (the Fab fragments) from the portion of the IgG molecule that binds to complement and Fc receptors (the Fc fragment).

Pepsin generates a single bivalent antigen-binding fragment, $F(ab')_2$. *N* and *C* at the ends of polypeptide chains refer to the amino and carboxy termini, respectively.

Structural Features of Antibody Variable Regions

Most of the sequence differences and variability among different antibodies are confined to three short stretches in the V region of the heavy chain and to three stretches in the V region of the light chain. These segments of the greatest diversity are known as **hypervariable regions.** They correspond to three protruding loops connecting adjacent strands of the β sheets that make up the V domains of Ig heavy and light chain proteins (Fig. 5.5). The hypervariable regions are each about 10 amino acid residues long, and they are held in place by the more conserved framework sequences that make up the Ig domain of the V region. Because these sequences form a surface that is complementary to the three-dimensional shape of the bound antigen, the hypervariable regions are also called **complementarity-determining regions (CDRs).** Proceeding from either the V_L or the V_H amino terminus, these regions are called CDR1, CDR2, and CDR3. Sequence differences among the CDRs of different antibody molecules contribute to distinct interaction surfaces and therefore to specificities of individual antibodies. The CDR3s of both the V_H segment and the V_L segment are the most variable of the CDRs. As we will discuss in Chapter 8, there are special mechanisms for generating more sequence diversity in CDR3 than in CDR1 and CDR2. In an antibody molecule, the three hypervariable regions of a V_L domain and the three hypervariable regions of a V_H domain are brought together to create an antigen-binding surface. The hypervariable loops can be thought to resemble fingers protruding from each variable domain, with three fingers from the heavy chain and three fingers from the light chain coming together to form the antigen-binding site (Fig. 5.6). The ability of a V region to fold into an Ig domain is mostly determined by the conserved sequences of the framework regions adjacent to the CDRs. Confinement of the sequence variability to three short stretches allows the basic structure of all antibodies to be maintained despite the variability that contributes to the specificities of different antibodies.

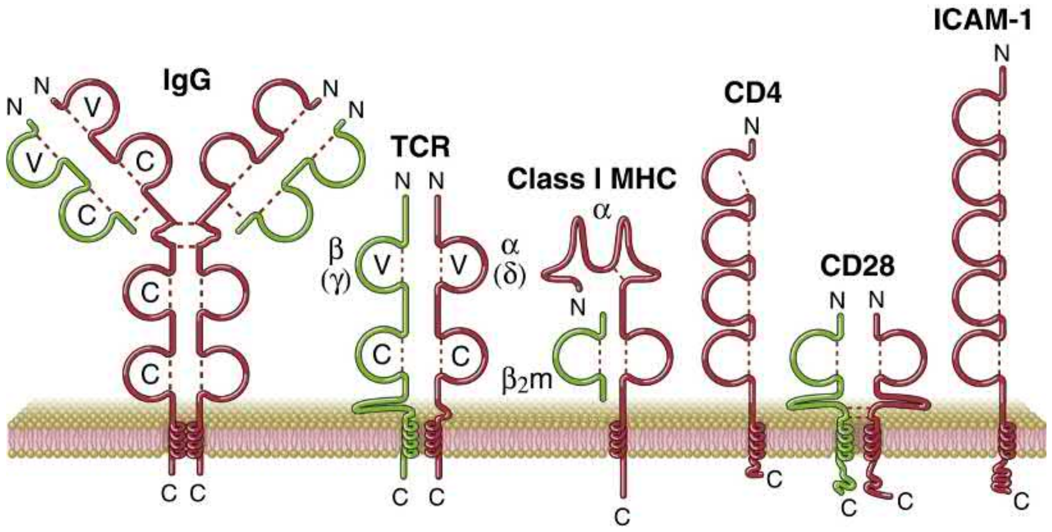


FIGURE 5.4 Examples of immunoglobulin (Ig) superfamily proteins in the immune system. Shown here are a membrane-bound IgG molecule; the T cell receptor; a major histocompatibility complex *(MHC)* class I molecule; the CD4 coreceptor of T cells; CD28, a costimulatory receptor on T cells; and the adhesion molecule *ICAM-1. N* and *C* at the ends of polypeptide chains refer to the amino and carboxy termini, respectively.

Crystallographic analyses of antigen-antibody complexes show that the amino acid residues of the CDRs form multiple contacts with bound antigens (see Fig. 5.6). The most extensive contact is with the third hypervariable region (CDR3). However, antigen binding is not solely a function of the CDRs, and framework residues also may contact the antigen. Moreover, in the binding of some antigens by specific antibody molecules, one or more of the CDRs may be outside the region of contact with the antigen, and therefore binding to certain antigens may occur independently of some of the Ig heavy or light chain CDRs.

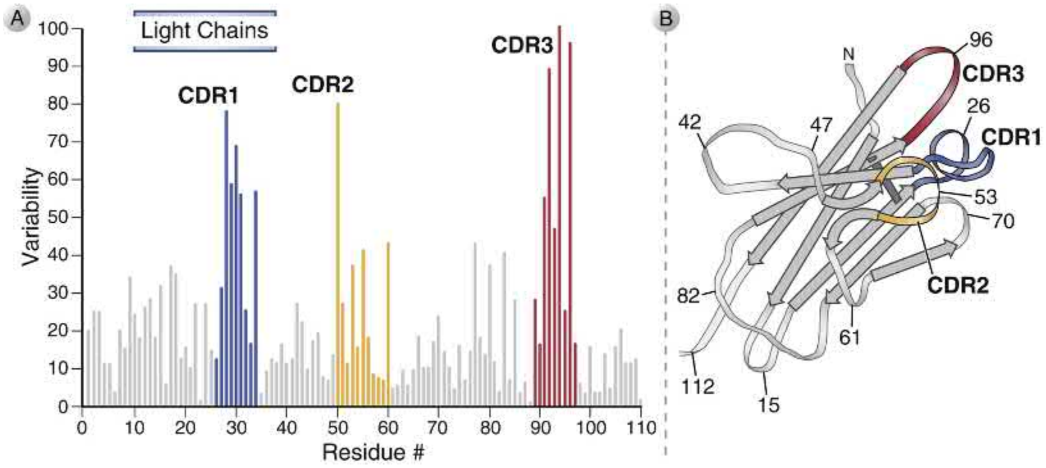


FIGURE 5.5 Hypervariable regions in immunoglobulin (Ig) molecules. **A,** The *vertical lines* depict the extent of variability, defined as the number of differences in each amino acid residue among various independently sequenced Ig light chains, plotted against amino acid residue number, measured from the amino terminus. This analysis indicates that the most variable residues are clustered in three “hypervariable” regions, colored *blue, yellow,* and *red,* corresponding to complementarity-determining regions (CDRs) CDR1, CDR2, and CDR3, respectively. Three hypervariable regions are also present in heavy chains (not shown). This way of displaying amino acid variability in Ig molecules is called a Kabat-Wu plot after the two scientists who devised the assay. **B,** Three-dimensional view of the hypervariable CDR loops in a light chain V domain. The V region of a light chain is shown with CDR1, CDR2, and CDR3 loops, colored *blue, yellow,* and *red,* respectively. These loops correspond to the hypervariable regions in the variability plot in **A.** Heavy chain hypervariable regions (not shown) are also located in three loops, and all six loops are juxtaposed in the antibody molecule to form the antigen-binding

surface (see Fig. 5.6). Note that in Fig. 5.2 an Ig constant domain, which does not have CDRs, is depicted.

A, Courtesy Dr. E.A. Kabat, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York.

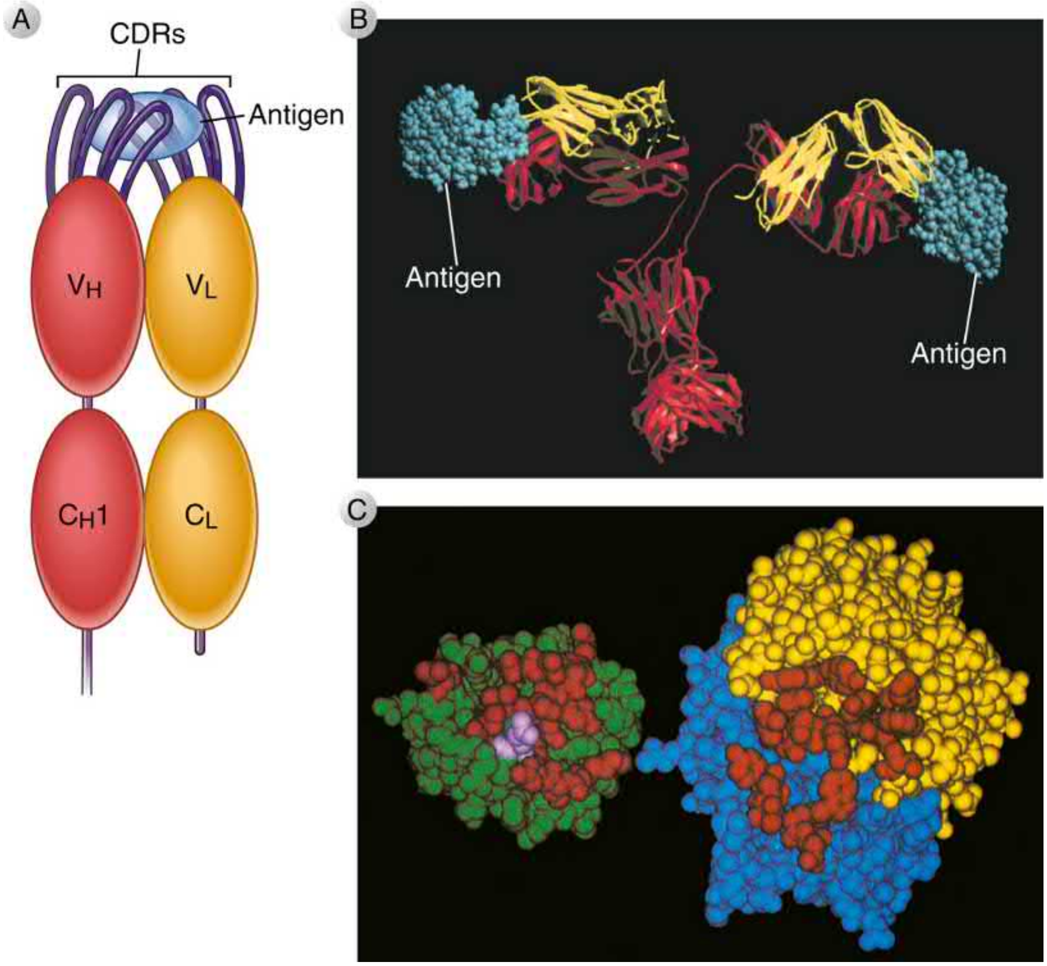


FIGURE 5.6 Binding of an antigen by an antibody. **A,** A schematic view of complementarity-determining regions *(CDRs)* generating an antigen-binding site. CDRs from the heavy chain and the light chain are loops that protrude from the surface of the two immunoglobulin V domains and in combination create an antigen-binding surface. **B,** This model of a globular protein antigen (hen egg lysozyme) bound to an antibody molecule shows how the antigen-binding site can accommodate soluble macromolecules in their native (folded) conformation. The heavy chains of the antibody are *red,* the light chains are *yellow,* and the antigen is *blue.* **C,** A view of the interacting surfaces of hen egg lysozyme (in

green) and a Fab fragment of a monoclonal anti–hen egg lysozyme antibody (V_H in *blue* and V_L in *yellow*) is provided. The residues of hen egg lysozyme and of the Fab fragment that interact with each other are shown in *red.* A critical glutamine residue on lysozyme (in *magenta*) fits into a "cleft" in the antibody.

B, Courtesy Dr. Dan Vaughn, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. *C,* Reprinted with permission from Amit AG, Mariuzza RA, Phillips SE, Poljak RJ. Three dimensional structure of an antigen antibody complex at 2.8A resolution. *Science.* 1986;233:747–753. Copyright 1986 by AAAS.

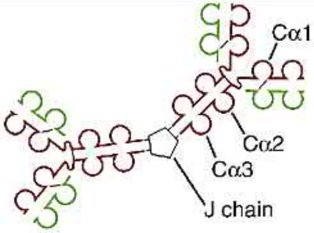
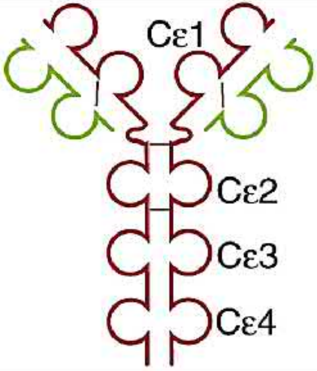
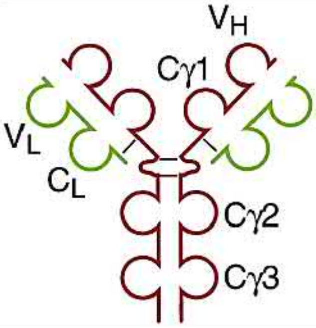
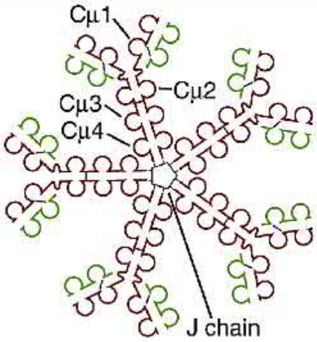
Structural Features of Antibody Constant Regions

Antibody molecules can be divided into distinct classes and subclasses on the basis of differences in the structure of their heavy chain C regions. The different antibody molecules are called **classes** or **isotypes** and are named IgA, IgD, IgE, IgG, and IgM (Table 5.2). In humans, IgA and IgG isotypes can be further subdivided into closely related subclasses, or subtypes, called IgA1 and IgA2 and IgG1, IgG2, IgG3, and IgG4. (Mice, which are often used in the study of immune responses, differ from humans in that the IgG isotype is divided into the IgG1, IgG2a, IgG2b, and IgG3 subclasses; certain strains of mice, including C57BL/6, lack the gene for IgG2a but produce a related isotype called IgG2c.) The heavy chain C regions of all antibody molecules of one isotype or subtype have essentially the same amino acid sequence. This sequence is different in antibodies of other isotypes or subtypes. Heavy chains are designated by the letter of the Greek alphabet corresponding to the isotype of the antibody: IgA1 contains $\alpha 1$ heavy chains; IgA2, $\alpha 2$; IgD, δ; IgE, ε; IgG1, $\gamma 1$; IgG2, $\gamma 2$; IgG3, $\gamma 3$; IgG4, $\gamma 4$; and IgM, μ. In human IgM and IgE antibodies, the C regions contain four tandem Ig domains (see Fig. 5.1). The C regions of IgG, IgA, and IgD contain only three Ig domains. These domains are generically designated C_H domains and are numbered sequentially from amino terminus to carboxy terminus (e.g., C_H1, C_H2, and so on). In each isotype, these regions may be designated more specifically (e.g., $C\gamma1$, $C\gamma2$ in IgG).

Different isotypes and subtypes of antibodies perform different effector functions. The reason for this is that most of the effector functions of antibodies are mediated by the binding of heavy chain C regions to Fc receptors (FcRs) on different cells, such as phagocytes, natural killer (NK) cells, and mast cells, and to plasma proteins, such as complement proteins. Antibody isotypes and subtypes differ in their C regions and therefore in what they bind to and what effector functions they perform. The effector functions mediated by each antibody isotype are listed in Table 5.2 and are discussed in more detail later in this chapter and in Chapter 13.

Antibody molecules are flexible, permitting them to bind to different arrays of antigens. Every antibody contains at least two antigen-binding sites, each formed by a pair of V_H and V_L domains. Many Ig molecules can orient these binding sites so that two antigen molecules on a planar (e.g., cell) surface may be engaged at once (Fig. 5.7). This flexibility is conferred, in large part, by the hinge region located between C_H1 and C_H2 in certain isotypes. The hinge region varies in length from 10 to more than 60 amino acid residues in different isotypes. Portions of this sequence assume an unfolded and flexible conformation, permitting molecular motion between the C_H1 and C_H2 domains. Some of the greatest differences between the constant regions of the IgG subclasses are concentrated in the hinge. This leads to different overall shapes of the IgG subtypes. In addition, some flexibility of antibody molecules is due to the ability of each V_H domain to rotate with respect to the adjacent C_H1 domain.

TABLE 5.2

Isotype of Antibody	Subtypes (H Chain)	Plasma Concentration (mg/mL)	Half-Life (Days)	Secreted Form		Functions[a]
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer; also monomer, trimer		Mucosal immunity
IgD	None (δ)	Trace	3	Monomer		B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer		Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	Monomer		Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer		Naive B cell antigen receptor (monomeric form), complement activation

Ig, Immunoglobulin.

[a] The effector functions of antibodies are discussed in detail in Chapter 13.

There are two classes, or isotypes, of light chains, called κ and λ, that have distinct carboxy-terminal constant (C) regions. Each antibody molecule has either two identical κ light chains or two identical λ light chains but never one of each. In humans, about 60% of antibody molecules have κ light chains and about 40% have λ light chains. Marked changes in this ratio can occur in patients with B cell tumors because the many neoplastic cells, being derived from one B cell clone, produce a single species of antibody molecules, all with the same light chain. In fact, an abnormal predominance of either κ-bearing cells or λ-bearing cells is often used clinically for the diagnosis of B cell lymphomas. In mice, κ-containing antibodies are about 10 times more abundant than λ-containing antibodies. Unlike in heavy chain isotypes, there are no known differences in function between κ-containing antibodies and λ-containing antibodies.

Secreted and membrane-associated antibodies differ in the amino acid sequence of the carboxy-terminal end of the heavy chain C region. The secreted form, found in blood, mucosal secretions, and other extracellular fluids, contains a carboxy-terminal hydrophilic region called the tail piece. The membrane-bound form of antibody contains a carboxy-terminal stretch that includes two segments: a hydrophobic α-helical transmembrane region, followed by an intracellular tail, which contains a juxtamembrane positively charged region of three amino acids (Fig. 5.8). The positively charged amino acids bind to negatively charged phospholipid head groups on the inner leaflet of the plasma membrane and help anchor the protein in the membrane, and are sometimes called a stop-transfer sequence. In membrane IgM and IgD molecules, the cytoplasmic portion of the heavy chain is short (only three amino acid residues in length). In membrane IgG and IgE molecules, the tail is approximately 30 amino acid residues in length and contains a signaling motif that contributes to memory B cell activation (see Chapters 7 and 12).

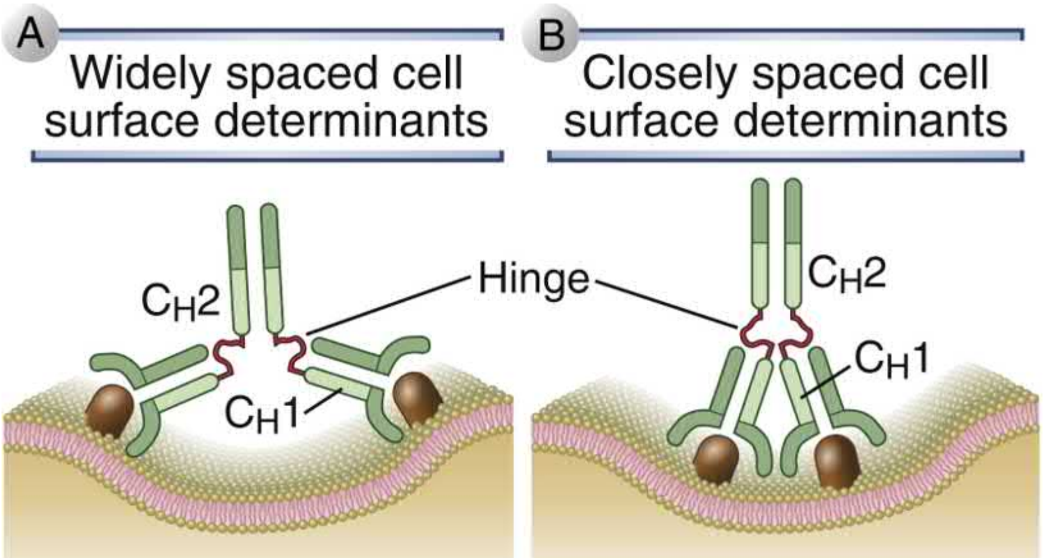

A
Widely spaced cell surface determinants
B
Closely spaced cell surface determinants
CH2
Hinge
CH1
CH2
CH1

FIGURE 5.7 Flexibility of antibody molecules. The two antigen-binding sites of an immunoglobulin (Ig) monomer can simultaneously bind to two determinants separated by varying distances. In **(A)** an Ig molecule is depicted binding to two widely spaced determinants on a cell surface, and in **(B)** the same antibody is binding to two determinants that are close together. This flexibility is mainly due to the hinge regions located between the C_H1 and C_H2 domains, which permit independent movement of antigen-binding sites relative to the rest of the molecule.

Secreted IgG and IgE and all membrane Ig molecules, regardless of isotype, are monomeric with respect to the basic antibody structural unit (i.e., they contain two heavy chains and two light chains). In contrast, the secreted forms of IgM and IgA form multimeric complexes in which two or more of the four-chain core antibody structural units are covalently joined. IgM is secreted mainly as pentamers but also some hexamers of the core four-chain structure, whereas IgA is usually secreted as a dimer. These complexes are formed by interactions between the tail pieces that are located at the carboxy-terminal ends of the secreted forms of μ and *α* heavy chains (see Table 5.2). Multimeric IgM and IgA molecules contain an additional non-Ig 15-kD polypeptide called the joining (J) chain, which is disulfide bonded to the tail pieces of the Ig C regions and serves to stabilize the multimeric complexes and to transport multimers across epithelial cells from the basolateral to the luminal end. As we will see later, multimeric forms of antibodies bind to antigens more avidly than monomeric forms.

Antibodies of different species differ from each other in the C regions and in framework parts of the V regions. Therefore, when Ig molecules from one species are introduced into another (e.g., horse serum antibodies or mouse monoclonal antibodies injected into humans), the recipient sees them as foreign, mounts an immune response, and makes antibodies largely against the C regions of the introduced Ig. The negative impact of this anti-Ig response on the use of antibodies as therapeutic agents is discussed in more detail later in the chapter.

Smaller sequence differences are present in antibodies from different individuals even of the same species, reflecting inherited polymorphisms in the genes encoding the C regions of Ig heavy and light chains. When polymorphic forms of an immunoglobulin found only in some individuals of a species can be recognized by antibodies, the sequences that differ among individuals are called **allotypes,** and the antibody that recognizes an allotypic variation is called an anti-allotypic antibody. Even in the same individual, differences among different antibodies that are concentrated in the CDRs constitute the **idiotypes** of antibodies. An antibody that recognizes some aspect of the CDRs of another antibody is therefore called an anti-idiotypic antibody. There have been interesting theories that individuals produce anti-idiotypic antibodies against their own antibodies that control immune responses, but there is little evidence to support the importance of this potential mechanism of immune regulation.

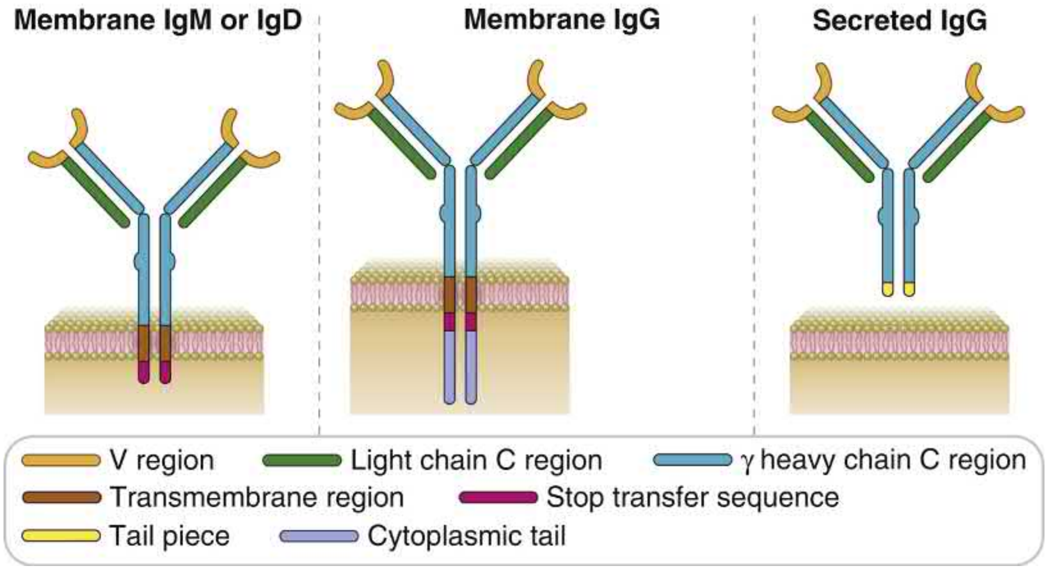

Membrane IgM or IgD
Membrane IgG
Secreted IgG
V region
Light chain C region
γ heavy chain C region
Transmembrane region
Stop transfer sequence
Tail piece
Cytoplasmic tail

FIGURE 5.8 Membrane and secreted forms of immunoglobulin (Ig) heavy chains.The membrane forms of the Ig heavy chains, but not the secreted forms, contain transmembrane regions made up of hydrophobic amino acid residues and cytoplasmic domains that differ significantly among the different isotypes. The cytoplasmic portion of the membrane form of the μ chain and the δ chain contains only three residues, whereas the cytoplasmic region of IgG and IgE membrane heavy chains (membrane γ and ε heavy chains) contains 20 to 30 residues. The secreted forms of the antibodies end in C-terminal tail pieces, which also differ among isotypes: μ has a long tail piece (21 residues) that is involved in pentamer formation, IgG has a short tail piece (3 residues).

Monoclonal Antibodies

Monoclonal antibodies are collections of identical antibodies. All molecules of a monoclonal antibody are produced by the progeny of a single B cell clone, and all the molecules therefore have the same V region and bind to the same antigen that originally triggered that B cell. A tumor of plasma cells (myeloma, or plasmacytoma), like most tumors of any cellular origin, is monoclonal and therefore produces antibodies of a single specificity. In most cases, the specificity of the tumor-derived antibody is not known, so the myeloma antibody cannot be used to detect or bind to molecules of interest. However, the discovery of monoclonal antibodies produced by these tumors led to the idea that it may be possible to produce similar monoclonal antibodies of any desired specificity by immortalizing individual antibody-secreting cells from an animal immunized with a known antigen. A technique to accomplish this was described by Georges Kohler and Cesar Milstein in 1975, and this has proved to be one of the most valuable advances in biology and medicine. The method relies on fusing B cells from an immunized animal (typically a mouse) with an immortal myeloma cell line and growing the cells under conditions in which only the fused normal and tumor cells can survive (Fig. 5.9). The resultant fused cells that grow out are called **hybridomas** because they are hybrids of normal B cells and a myeloma tumor. Each hybridoma makes only one Ig, derived from one B cell from the immunized animal. The antibodies secreted by many hybridoma clones are screened for binding to the antigen of interest, and the clone with the desired specificity is selected and expanded. The products of these individual clones are monoclonal antibodies, and each monoclonal antibody is specific for a single epitope on the antigen used to immunize the animal.

Monoclonal antibodies have many applications in research and in medical diagnosis and therapy. Some of their common applications include the following:

- ***Identification of phenotypic markers unique to particular cell types.*** The basis for the modern classification of lymphocytes and other leukocytes is the recognition of individual cell populations by specific monoclonal antibodies, most often by using flow cytometry and related techniques (see Appendix III). These antibodies have been used to define clusters of differentiation (CD) markers for various cell types (see Chapter 2 and Appendix I).

- ***Immunodiagnosis.*** The diagnosis of many infectious and systemic diseases, and even of pregnancy, relies on the detection of particular antigens or antibodies in the blood, urine, or tissues by use of monoclonal antibodies in immunoassays (see Appendix III).
- ***Tumor identification.*** Labeled monoclonal antibodies specific for various cell proteins are used to determine the tissue source of tumors by staining histologic tumor sections.
- ***Therapy.*** Advances in medical research have led to the identification of cells and molecules that are involved in the pathogenesis of many diseases. Monoclonal antibodies, because of their exquisite specificity, provide a means of targeting these cells and molecules. Many monoclonal antibodies are used therapeutically today (Table 5.3). Some examples include antibodies specific for the cytokine tumor necrosis factor (TNF) used to treat rheumatoid arthritis and other inflammatory diseases, antibodies against CD20 for the treatment of B cell–derived tumors and for depleting B cells in certain autoimmune disorders, antibodies specific for the T cell regulatory molecules PD-1 and CTLA-4 used in therapy for many types of cancers, antibodies that bind to epidermal growth factor receptors to target cancer cells, antibodies against vascular endothelial growth factor (a cytokine that promotes angiogenesis) in patients with macular degeneration, and so on.
- ***Functional analysis of cell surface and secreted molecules.*** In biologic research, monoclonal antibodies that bind to cell surface molecules and either stimulate or inhibit particular cellular functions are invaluable tools for defining the functions of these molecules, including receptors for antigens. Monoclonal antibodies are also widely used to purify selected cell populations from complex mixtures to facilitate the analysis of the properties and functions of

these cells, and to block or deplete secreted molecules and particular cells for studying their functions.

One of the limitations of monoclonal antibodies for therapy is that these antibodies are most easily produced by immunizing mice, but patients treated with mouse antibodies will make antibodies against the mouse Ig, called human antimouse antibody (HAMA). These anti-Ig antibodies block the function or enhance clearance of the injected monoclonal antibody and also can cause serum sickness (see Chapter 19). Genetic engineering techniques have been used to replace the mouse sequences in the monoclonal antibody with human sequences and thereby avoid an anti-Ig response. The complementary DNAs (cDNAs) that encode the polypeptide chains of a monoclonal antibody can be isolated from a hybridoma, and these genes can be manipulated in vitro. As discussed earlier, only small portions of the antibody molecule are responsible for binding to antigen; the remainder of the antibody molecule can be thought of as a framework. This structural organization allows the DNA segments encoding the antigen-binding sites from a mouse monoclonal antibody to be inserted into a cDNA encoding a human myeloma protein, creating a hybrid gene. When it is expressed, the resultant protein, which retains the antigen specificity of the original mouse monoclonal but has the core structure of a human Ig, is referred to as a humanized antibody. Fully human monoclonal antibodies are also in clinical use. These are derived using phage display methods or in mice with B cells expressing human Ig transgenes. The **phage display** technology for generating monoclonal antibodies against specific targets involves screening bacteriophage libraries in which each bacteriophage displays on its surface one synthetically created antigen-binding site of human Ig. By screening millions of such synthetic antibody-binding sites for binding to the antigen of interest, one that is specific for the target antigen can be identified.

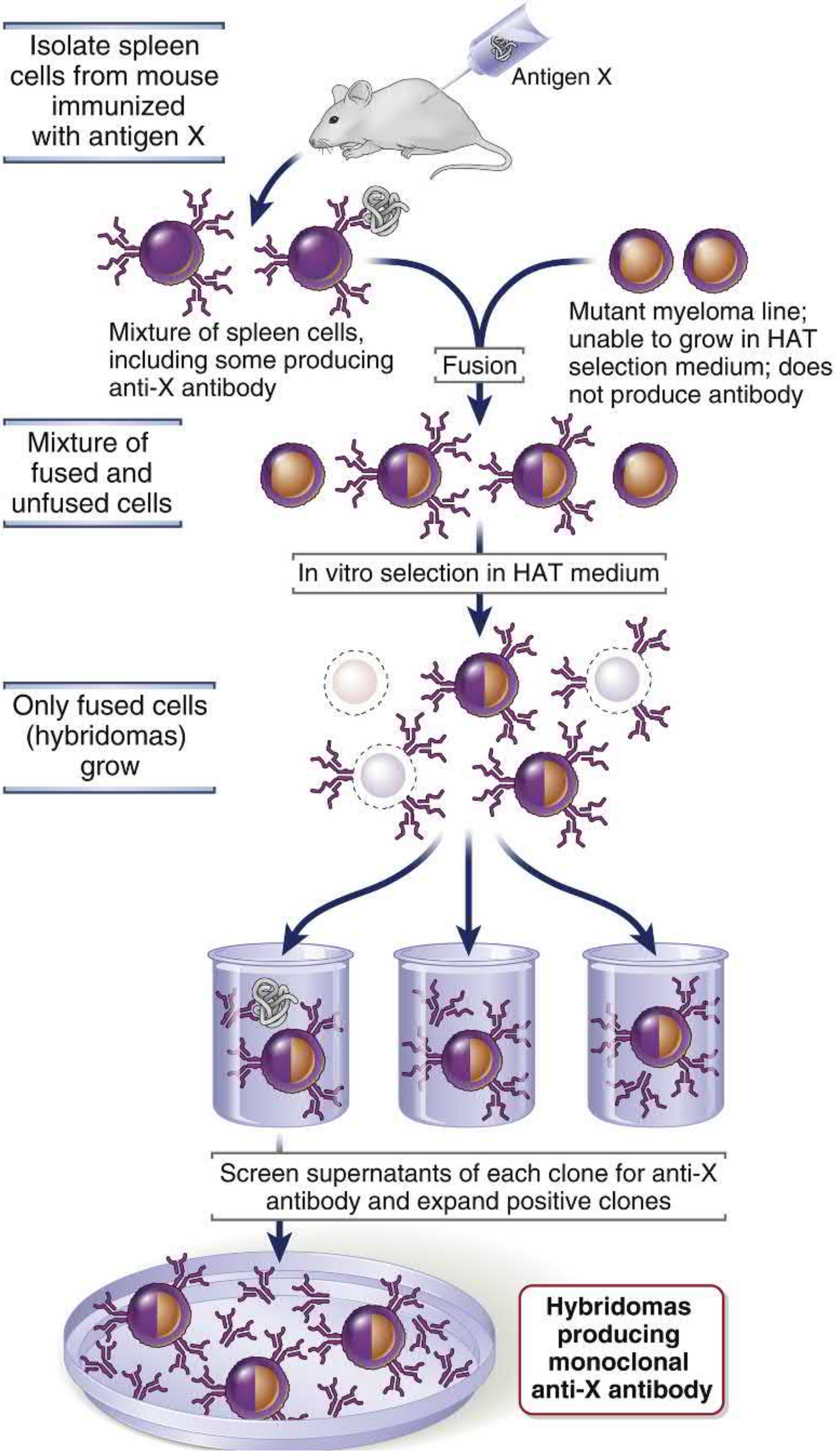

Isolate spleen cells from mouse immunized with antigen X
Antigen X
Mixture of spleen cells, including some producing anti-X antibody
Fusion
Mutant myeloma line; unable to grow in HAT selection medium; does not produce antibody
Mixture of fused and unfused cells
In vitro selection in HAT medium
Only fused cells (hybridomas) grow
Screen supernatants of each clone for anti-X antibody and expand positive clones
Hybridomas producing monoclonal anti-X antibody

FIGURE 5.9 The generation of monoclonal antibodies. In this procedure, spleen B cells from a mouse that has been immunized with a known antigen or mixture of antigens are fused with an enzyme-deficient partner myeloma cell line, with use of chemicals such as polyethylene glycol that can facilitate the fusion of plasma membranes and the formation of hybrid cells that retain many chromosomes from both fusion partners. The myeloma partner used is one that does not secrete its own immunoglobulin (Ig). These hybrid cells are then placed in a selection medium that permits the survival of only immortalized hybrids; these hybrid cells are then grown as single cell clones and tested for the secretion of the antibody of interest. The selection medium includes hypoxanthine, aminopterin, and thymidine and is therefore called HAT medium. There are two pathways of purine synthesis in most cells, a de novo pathway that needs tetrahydrofolate and a salvage pathway that uses the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Myeloma cells that lack HGPRT are used as fusion partners, and they normally survive using de novo purine synthesis. In the presence of aminopterin, tetrahydrofolate is not made, resulting in a defect in de novo purine synthesis and also a specific defect in pyrimidine biosynthesis, namely, in generating thymidine monophosphate (TMP) from deoxyuridine monophosphate (dUMP). Hybrid cells receive HGPRT from the splenic B cells and acquire the capacity for uncontrolled proliferation from the myeloma partner; if they are given hypoxanthine and thymidine, these cells can make DNA in the

absence of tetrahydrofolate. As a result, only hybrid cells survive in HAT medium.

TABLE 5.3

Target	Effect	Diseases
Inflammatory (Immunologic) Diseases		
α4 Integrins	Blocking of immune cell egress to intestine and CNS	Crohn's disease, multiple sclerosis
CD20	Depletion of B cells	Rheumatoid arthritis, multiple sclerosis, other autoimmune diseases, B cell lymphomas
IgE	Blocking of IgE function	Allergy-related asthma
TNF	Reducing inflammation	Rheumatoid arthritis, Crohn's disease, psoriasis
IL-17	Reducing inflammation	Psoriasis
Other Diseases		
C5	Blocking of complement-mediated lysis	Paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome
Glycoprotein IIb/IIIa	Inhibition of platelet aggregation	Cardiovascular disease
RANK ligand	Blocking of RANK signaling	Postmenopausal osteoporosis, bone metastases of solid tumors
SARS-CoV-2 proteins	Blocking of viral entry	COVID-19
Cancer (see Table 18.1, Chapter 18)		

CNS, Central nervous system; *IgE,* immunoglobulin E; *IL,* interleukin; *RANK,* receptor activator of nuclear factor-κB; *RSV,* respiratory syncytial virus; *TNF,* tumor necrosis factor.

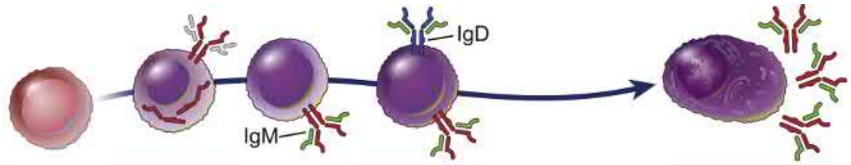
[a] Additional anticytokine antibodies in clinical use are listed in Table 19.5, Chapter 19.

Humanized antibodies are far less likely than mouse monoclonals to appear foreign in humans and to induce anti-antibody responses. However, a proportion of subjects receiving fully humanized monoclonal antibodies for therapy develop blocking anti-antibodies, known as human-antihuman antibodies (HAHAs). Why this happens in some individuals is not understood.

Synthesis, Assembly, and Expression of Immunoglobulin Molecules

Immunoglobulin heavy and light chains, like most secreted and membrane proteins, are synthesized on membrane-bound ribosomes in the rough endoplasmic reticulum. The protein is translocated into the endoplasmic reticulum, and Ig heavy chains are *N*-glycosylated during the translocation process. The proper folding of Ig heavy chains and their assembly with light chains are regulated by proteins resident in the endoplasmic reticulum called chaperones. These proteins, which include calnexin and a molecule called binding protein (BiP), bind to newly synthesized Ig polypeptides and ensure that they are retained or targeted for degradation unless they fold properly and assemble into complete Ig molecules. The covalent association of heavy and light chains is stabilized by the formation of disulfide bonds, which also occurs in the endoplasmic reticulum during the assembly process. After assembly, the Ig molecules are released from chaperones, transported into the cisternae of the Golgi complex, where carbohydrates are modified, and then routed to the plasma membrane in vesicles. Antibodies of the membrane form are anchored in the plasma membrane, and the secreted form is transported out of the cell.

The maturation of B cells from bone marrow progenitors is accompanied by specific changes in Ig gene expression, resulting in the production of Ig molecules in different forms (Fig. 5.10) **.** The earliest cell in the B lymphocyte lineage that produces Ig polypeptides, called the pre-B cell, synthesizes the membrane form of the μ heavy chain. These μ chains associate with proteins called surrogate light chains to form the pre-B cell receptor, which is expressed on the cell surface and provides signals that promote maturation. Immature and mature B cells produce κ or λ light chains, which associate with μ proteins to form IgM molecules. Mature B cells express membrane forms of IgM and IgD (the μ and δ heavy chains associated with κ or λ light chains). These membrane Ig receptors serve as cell surface receptors that recognize antigens and initiate the process of B cell activation. The pre-B cell receptor and the B cell antigen receptor are noncovalently associated with two other integral membrane proteins, Igα and Igβ, which serve signaling functions and are essential for surface expression of IgM and IgD. We will discuss the molecular and cellular events in B cell maturation underlying these changes in antibody expression in Chapter 8.



Stage of maturation	Stem cell	Pre-B cell	Immature B cell	Mature B cell	Activated B cell (plasmablast)	Plasma cell
Pattern of immunoglobulin production	None	Cytoplasmic µ heavy chain and pre-B receptor	Membrane IgM	Membrane IgM, IgD	Low rate Ig secretion; heavy chain isotype switching; affinity maturation	High rate Ig secretion; little or no membrane Ig

FIGURE 5.10 Immunoglobulin (Ig) expression during B lymphocyte maturation. Stages in B lymphocyte maturation are shown with associated changes in the production of Ig heavy and light chains. IgM heavy chains are shown in *red,* IgD heavy chains in *blue,* and light chains in *green.* The molecular events accompanying these changes are discussed in Chapters 8 and 12.

When mature B lymphocytes are activated by antigens and other stimuli, the cells differentiate into plasmablasts and then into antibody-secreting plasma cells. This process is also accompanied by changes in the pattern of Ig production. One such change is increased production of the secreted form of Ig relative to the membrane form. This alteration occurs at the level of post-transcriptional processing. The second change is the expression of Ig heavy chain isotypes other than IgM and IgD, by a process called heavy chain isotype (or class) switching. A third change involves the introduction of new amino acid substitutions into the variable domains of the antibody heavy and light chains to create high-affinity antibodies, resulting in a change in antibodies that is called affinity maturation. Changes in antibody expression that occur after B cell activation will be discussed later in this chapter and in more detail in Chapter 12.

Half-Life of Antibodies

The half-life of circulating antibodies is a measure of how long those antibodies remain in blood after secretion from B cells (or after injection in the case of an administered antibody). The half-life is the mean time before the number of antibody molecules is reduced by half. Different antibody isotypes have very different half-lives in circulation. IgE has a very short half-life of about 2 days in the circulation (although cell-bound IgE associated with the high-affinity IgE receptor on mast cells has a very long half-life; see Chapter 20). Circulating IgA has a half-life of about 3 days (although most IgA is produced at mucosal sites and is secreted directly into the lumen of the gut or airway), and circulating IgM has a half-life of about 4 days. In contrast, circulating IgG molecules have a half-life of about 21 to 28 days.

The long half-life of IgG is attributed to its ability to bind to a specific Fc receptor called the **neonatal Fc receptor (FcRn),** so-called because it is involved in the transport of IgG from the maternal circulation into the fetus. FcRn structurally resembles MHC class I molecules (described in Chapter 6). It is expressed in the placenta, where it transports IgG molecules from the maternal blood, across cells, and into the fetal circulation (see Chapter 14). In adult vertebrates, FcRn is found on endothelial cells, macrophages, and other cell types, and binds to IgG that is ingested from the blood by pinocytosis into endosomes. The IgG remains bound to FcRn in the acidic environment of the endosomes. FcRn does not target the bound IgG to lysosomes (the usual fate of many ingested molecules) but recycles it to the cell surface and releases it in the neutral pH of the blood, returning the IgG to the circulation (Fig. 5.11). This intracellular sequestration of IgG away from lysosomes prevents the IgG from being degraded as rapidly as most other plasma proteins, including other antibody isotypes, and as a result the IgG isotype has a relatively long half-life. There are some differences in the half-lives of the four human IgG subtypes. IgG3 is relatively short-lived because it binds poorly to FcRn. IgG1, IgG2, and IgG4 are long-lived.

The long half-life of IgG has been used to provide a therapeutic advantage for certain injected proteins by producing fusion proteins containing the biologically active part of the protein and the Fc portion of IgG. The Fc portion enables the proteins to bind to the FcRn and thus extends the half-lives of the injected proteins. One therapeutically useful Fc fusion protein is TNFR-Ig, which consists of the extracellular domain of the type II TNF receptor (TNFR) fused to an IgG Fc domain. TNFR-Ig binds to TNF and blocks its inflammatory actions, similar to an anti-TNF antibody, and is used to treat certain immune disorders such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis (Fig. 5.12). Another Fc fusion protein used therapeutically is CTLA4-Ig, containing the extracellular domain of the CTLA-4 receptor, which binds to and blocks B7 costimulators, fused to the Fc portion of human IgG; it has also been used in the treatment of rheumatoid arthritis and kidney transplant rejection.

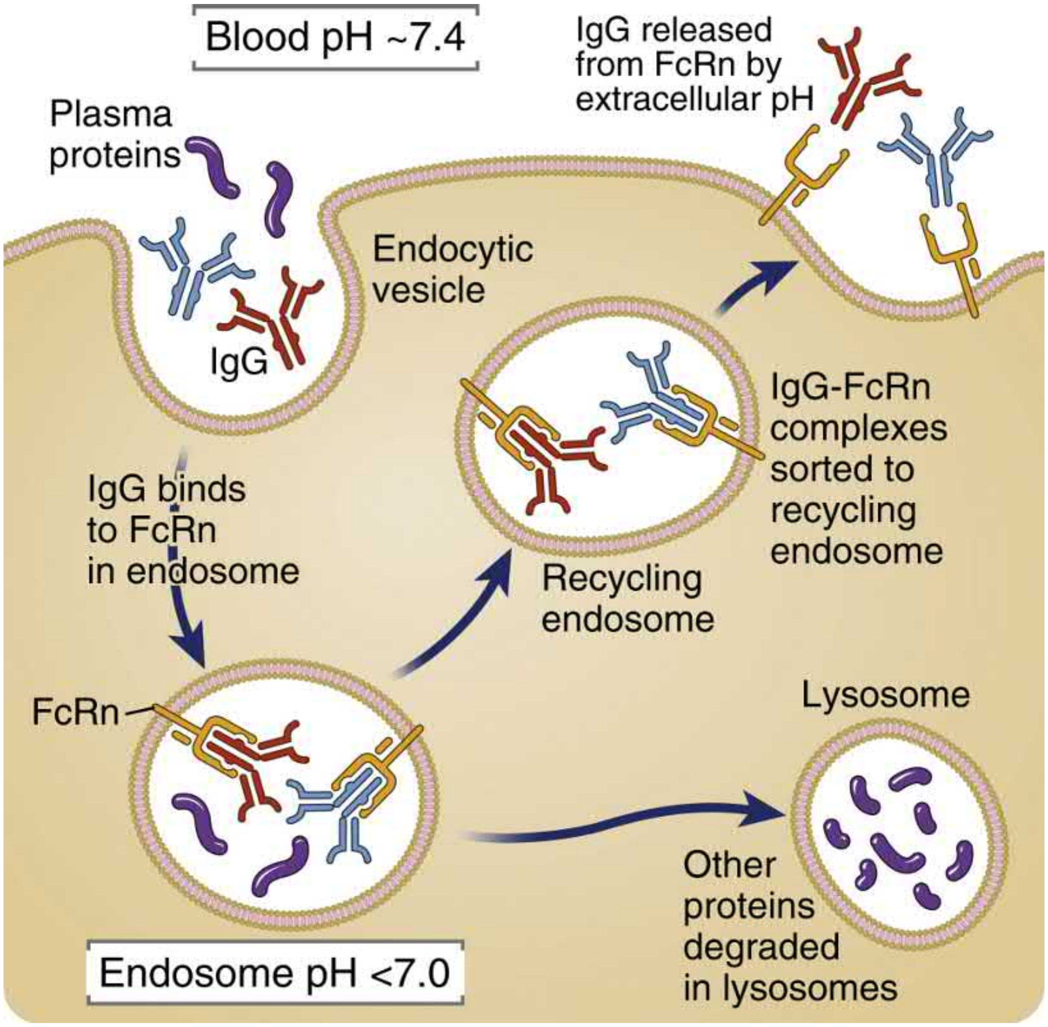

Blood pH ~7.4
IgG released from FcRn by extracellular pH
Plasma proteins
Endocytic vesicle
IgG
IgG-FcRn complexes sorted to recycling endosome
IgG binds to FcRn in endosome
Recycling endosome
Lysosome
FcRn
Other proteins degraded in lysosomes
Endosome pH <7.0

FIGURE 5.11 Neonatal Fc receptor (FcRn) contributes to the long half-life of immunoglobulin G (IgG) molecules.Micropinocytosed IgG molecules in endothelial cells bind the FcRn, an IgG-binding receptor in the acidic environment of endosomes. In endothelial cells, FcRn directs the IgG molecules away from lysosomal degradation and releases them when vesicles fuse with the cell surface, exposing FcRn-IgG complexes to neutral pH.

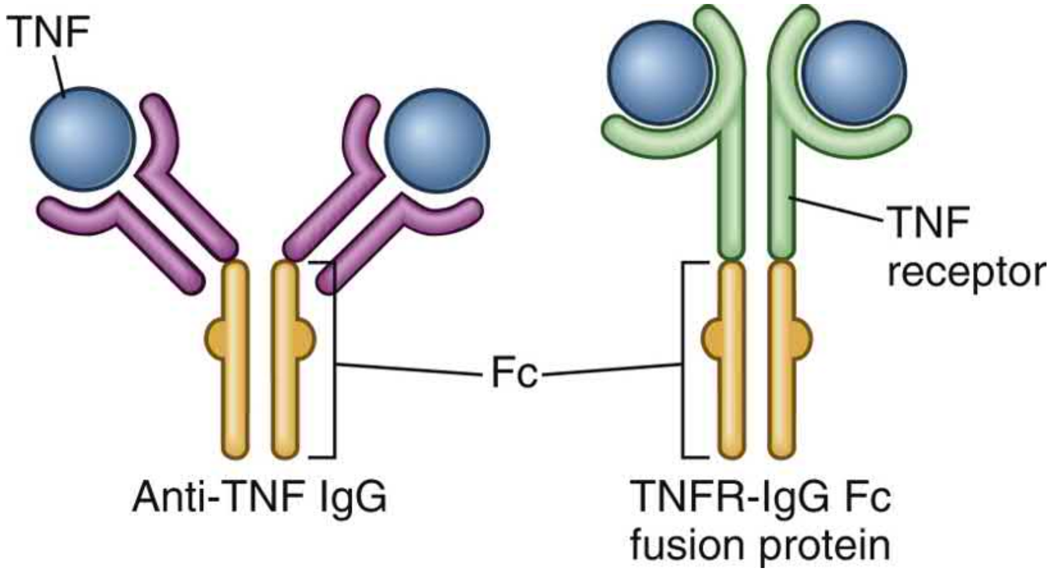


FIGURE 5.12 A monoclonal antibody and a cytokine receptor–immunoglobulin G (IgG) Fc fusion protein, both used therapeutically.An antibody specific for the cytokine tumor necrosis factor *(TNF) (left)* can bind to and block the activity of the cytokine. The extracellular domain of the TNF receptor *(right)* is also an antagonist of the cytokine, and linking this soluble receptor domain to an IgG Fc domain (by recombinant DNA technology) increases the half-life of the receptor in the circulation.

Antibody Binding of Antigens

All of the functions of antibodies depend on their ability to specifically bind antigens. We will now consider the nature of antigens and how they are recognized by antibodies.

Features of Biologic Antigens

An antigen is any substance that may be specifically bound by an antibody molecule or TCR. The antigens that are recognized by antibodies may be almost every kind of biologic molecule, including simple intermediary metabolites, sugars, lipids, autacoids, and hormones, as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acids, and proteins. This is in contrast to most T cells, which only recognize peptides (see Chapter 6).

Not all antigens recognized by specific lymphocytes or by secreted antibodies are capable of activating lymphocytes. Molecules that stimulate immune responses are called **immunogens.** Macromolecules are effective at stimulating B lymphocytes to initiate humoral immune responses because B cell activation requires the bringing together (cross-linking) of multiple antigen receptors. Small chemicals, such as dinitrophenol, may bind to antibodies and are therefore antigens, but they cannot activate B cells on their own because they can bind to only one B cell receptor at a time. To generate antibodies specific for such small chemicals, immunologists commonly attach multiple copies of the small molecules to a protein or polysaccharide before immunization. In these cases, the small chemical is called a **hapten,** and the large molecule to which it is conjugated is called a **carrier.** The hapten-carrier complex, unlike free hapten, can bind to multiple antigen receptors on a B cell and hence act as an immunogen. This phenomenon has been exploited to produce effective vaccines (see Chapter 12).

Macromolecules, such as proteins, polysaccharides, and nucleic acids, are usually much larger than the antigen-binding region of an antibody molecule (see Fig. 5.6). Therefore, any antibody binds to only a portion of the macromolecule, which is called a **determinant** or an **epitope.** These two words are synonymous and are used interchangeably throughout this book. The portion of the antibody that directly interacts with the epitope has been called the paratope. Macromolecules typically contain multiple determinants, some of which may be repeated and each of which, by definition, can be bound by an antibody. The presence of multiple identical determinants in an antigen is referred to as **polyvalency** or **multivalency.** Most globular proteins do not contain multiple identical determinants and are not individually polyvalent, but many identical proteins may be displayed in a polyvalent array on cell surfaces, including the surface of microbes. In the case of polysaccharides and nucleic acids, many identical epitopes may be regularly spaced and repeated in the same molecule, and these molecules are said to be polyvalent. Polyvalent arrays of carbohydrate antigens also can be displayed on cell surfaces. Polyvalent antigens can induce clustering of the B cell receptor and thus initiate the process of B cell activation (see Chapter 12).

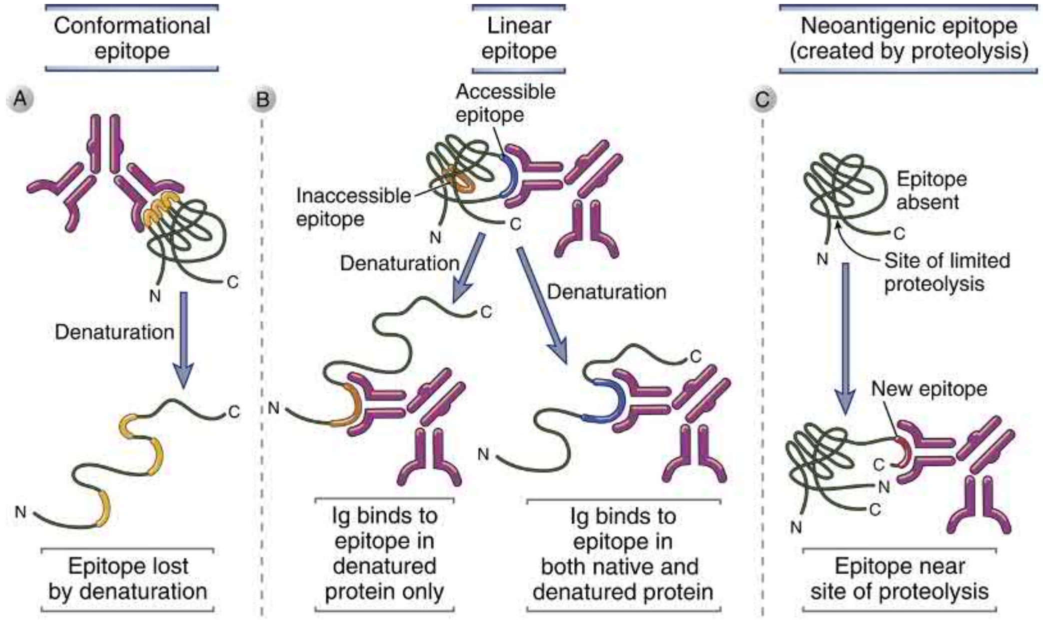

Conformational epitope
Linear epitope
Neoantigenic epitope (created by proteolysis)
A
B
C
Accessible epitope
Inaccessible epitope
N
C
Denaturation
Denaturation
Denaturation
Epitope absent
Site of limited proteolysis
New epitope
Epitope lost by denaturation
Ig binds to epitope in denatured protein only
Ig binds to epitope in both native and denatured protein
Epitope near site of proteolysis

FIGURE 5.13 The nature of antigenic determinants.Antigenic determinants (shown in *orange, red,* and *blue*) may depend on protein folding (conformation) and on primary structure. Some determinants are accessible in native proteins and are lost on denaturation **(A),** whereas others are exposed only on protein unfolding **(B).** Neodeterminants arise from postsynthetic modifications such as peptide bond cleavage **(C).** *N* and *C* at the ends of polypeptide chains refer to the amino and carboxy termini, respectively.

The spatial arrangement of different epitopes on a single protein molecule may influence the binding of antibodies in several ways. When determinants are well separated, two or more antibody molecules can be bound to the same protein antigen without influencing each other; such determinants are said to be nonoverlapping. When two determinants are close to each other, the binding of antibody to the first determinant may cause steric interference with the binding of antibody to the second; such determinants are said to be overlapping. In rare cases, the binding of one antibody may cause a conformational change in the structure of the antigen, positively or negatively influencing the binding of a second antibody at another site on the protein by means other than steric hindrance. Such interactions are called allosteric effects.

Any available shape or surface on a molecule that is recognized by an antibody constitutes an antigenic determinant or epitope. Antigenic determinants may be present on any type of molecule, including but not restricted to carbohydrates, proteins, lipids, and nucleic acids. In the case of proteins, the formation of some epitopes depends only on the primary structure, and the formation of other determinants reflects tertiary structure, or conformation (shape) (Fig. 5.13). Epitopes formed by several adjacent amino acid residues are called linear epitopes. The antigen-binding site of an antibody can usually accommodate a linear epitope made up of about six amino acids. If linear epitopes appear on the external surface or in a region of extended conformation in the native folded protein, they may be accessible to antibodies. In other cases, linear epitopes may be inaccessible in the native conformation and appear only when the protein is denatured. In contrast, conformational epitopes are formed by amino acid residues that are not in a sequence but become spatially juxtaposed in the folded protein. Antibodies specific for certain linear epitopes and antibodies specific for conformational epitopes can be used to ascertain whether a protein is denatured or in its native conformation, respectively. Proteins may be subjected to modifications such as glycosylation, phosphorylation, ubiquitination, acetylation, and proteolysis. These modifications, by altering the structure of the protein, can produce new epitopes. New epitopes also may be generated in a tumor by mutations of genes encoding self proteins. Such epitopes, induced by post-translational modifications or by mutation, are called neoantigenic epitopes, and they too may be recognized by specific antibodies (and T cells).

Structural and Chemical Basis of Antigen Binding

The antigen-binding sites of many antibodies are planar surfaces that can accommodate conformational epitopes of macromolecules, allowing the antibodies to bind large macromolecules (see Fig. 5.6) **.** The six CDRs, three from the heavy chain and three from the light chain, can spread out to form a broad surface. In a number of antibodies specific for small molecules, such as monosaccharides and drugs, the antigen is bound in a cleft generated by the close apposition of CDRs from the V_L and V_H domains.

The recognition of antigens by antibodies involves noncovalent, reversible binding. Various types of noncovalent interactions may contribute to antibody binding of antigens, including electrostatic forces, hydrogen bonds, van der Waals forces, and hydrophobic interactions. The relative importance of each of these depends on the structures of the binding site of the individual antibody and of the antigenic determinant. The strength of the binding between a single combining site of an antibody and an epitope of an antigen is called the **affinity** of the antibody. The affinity is commonly represented by a dissociation constant (K_d), which indicates how easy it is to separate (dissociate) an antigen-antibody complex into its constituents by changing their concentration. A smaller K_d indicates a stronger or higher affinity interaction because a lower concentration of antigen and of antibody is required for complex formation. The K_d of antibodies produced in typical humoral immune responses usually varies from about 10^{-7} to 10^{-11} M. Serum from an immunized individual will contain a mixture of antibodies with different affinities for the antigen.

Because the hinge region of antibodies gives them flexibility, a single antibody may attach to a single multivalent antigen by more than one binding site. For IgG or IgE, this attachment can involve, at most, two binding sites, one on each Fab. For pentameric IgM, however, a single antibody may bind at up to 10 different sites (Fig. 5.14). Polyvalent antigens will have more than one copy of a particular determinant. Although the affinity of any one antigen-binding site will be the same for each epitope of a polyvalent antigen, the strength of attachment of the antibody to the antigen must take into account all the binding sites of the antibody and all the available epitopes of the antigen. This overall strength of attachment is called the **avidity** and is much greater than the affinity of any one antigen-binding site. Thus, a low-affinity IgM molecule can still bind tightly to a polyvalent antigen because many low-affinity interactions (up to 10 per IgM molecule) can produce a high-avidity interaction.

Polyvalent antigens are important from the viewpoint of B cell activation, as discussed earlier. Polyvalent interactions between antigens and antibodies are also of biologic significance because many effector functions of antibodies are triggered optimally when two or more antibody molecules are brought close together by binding to a polyvalent antigen. If a polyvalent antigen is mixed with a specific antibody in a test tube, the two interact to form **immune complexes** (Fig. 5.15). At the correct concentration, called a zone of equivalence, antibody and antigen form an extensively cross-linked network of attached molecules such that most or all of the antigen and antibody molecules are complexed into large masses. Immune complexes may be dissociated into smaller aggregates either by increasing the concentration of antigen so that free antigen molecules will displace antigen bound to the antibody (zone of antigen excess) or by increasing antibody so that free antibody molecules will displace bound antibody from antigen determinants (zone of antibody excess). If a zone of equivalence is reached in vivo, large immune complexes can form in the circulation. Immune complexes that are trapped or formed in the walls of blood vessels can initiate an inflammatory reaction, resulting in immune complex diseases (see Chapter 19).

Structure-Function Relationships in Antibody Molecules

Many structural features of antibodies are critical for their ability to recognize antigens and for their effector functions. In the following section, we will summarize how the structure of antibodies contributes to their functions.

Features Related to Antigen Recognition

The ability of antibodies to specifically recognize a wide variety of antigens with varying affinities reflects the properties of the V regions.

Specificity

Antibodies can be remarkably specific for antigens, distinguishing between small differences in chemical structure. The fine specificity of antibodies applies to the recognition of all classes of molecules. For example, antibodies can distinguish between two linear protein determinants differing by only a single conservative amino acid substitution that has little effect on secondary structure. This high degree of specificity is necessary so that antibodies generated in response to the antigens of one microbe usually do not react with structurally similar self molecules or with the antigens of other microbes. However, some antibodies produced against one antigen may bind to a different but structurally related antigen. This is referred to as a **cross-reaction.** Antibodies that are produced in response to a microbial antigen sometimes cross-react with self antigens, and this may be the basis of certain immunologic diseases (see Chapter 19).

Diversity

As we discussed earlier in this chapter, an individual is capable of making a tremendous number of structurally distinct antibodies, on the order of millions, each with a distinct specificity. The ability of antibodies in any individual to specifically bind a large number of different antigens is a reflection of antibody **diversity,** and the total collection of antibodies with different specificities represents the antibody **repertoire.** The genetic mechanisms that generate such a large antibody repertoire are active only in B lymphocytes (and the same mechanisms for generating TCR diversity are active in T cells). This diversity is generated by random recombination of a limited set of inherited germline DNA sequences to form functional genes that encode the V regions of heavy and light chains as well as by the addition of nucleotide sequences during the recombination process. We will discuss these mechanisms in detail in Chapter 8. The millions of resulting variations in structure are concentrated in the antigen-binding hypervariable regions of both heavy and light chains and thereby determine specificity for antigens.

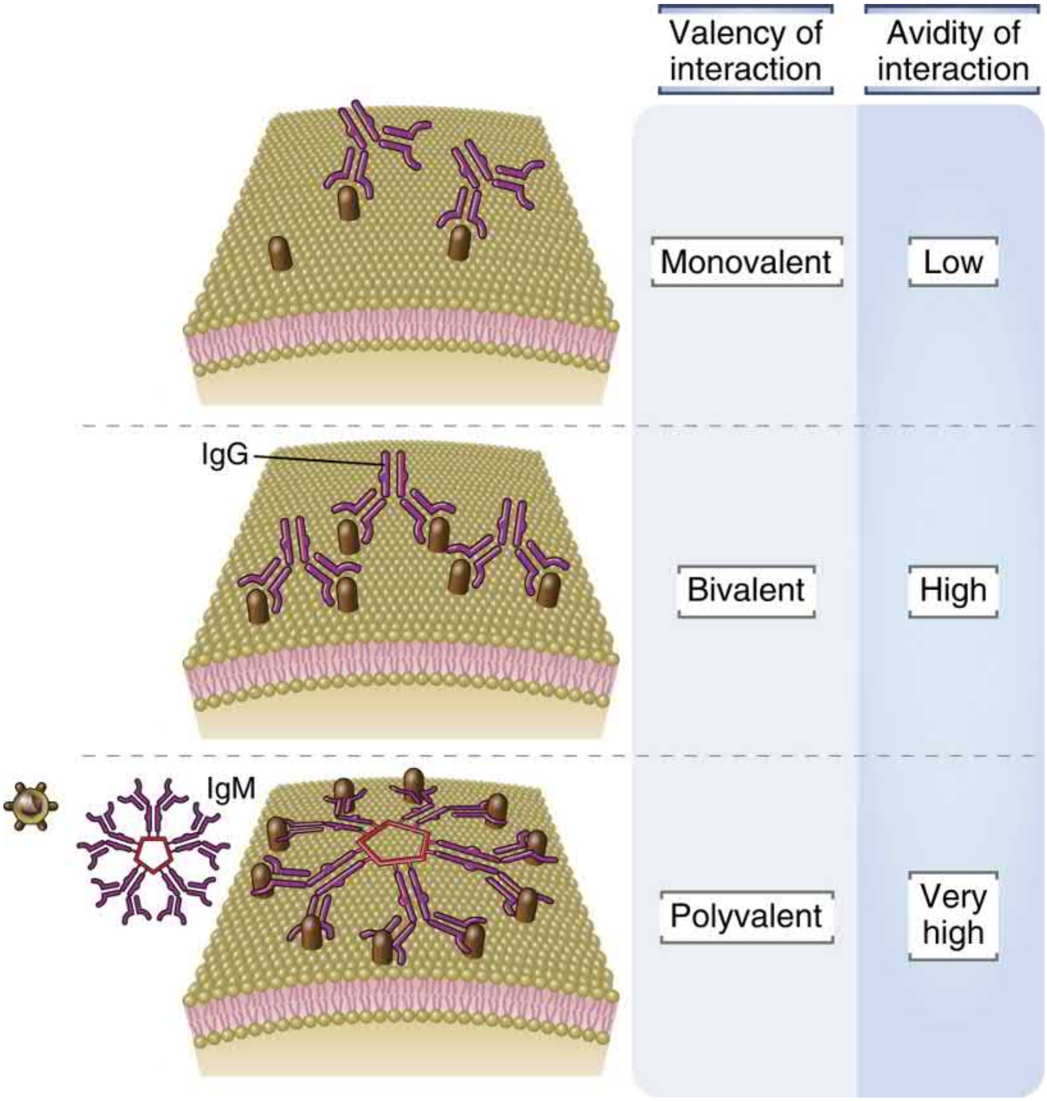

Valency of interaction
Avidity of interaction
Monovalent
Low
IgG
Bivalent
High
IgM
Polyvalent
Very high

FIGURE 5.14 Valency and avidity of antibody-antigen interactions.Monovalent antigens, or epitopes spaced far apart on cell surfaces, will interact with a single binding site of one antibody molecule. Although the affinity of this interaction may be high, the overall avidity may be relatively low. When repeated determinants on a cell surface are close enough, both the antigen-binding sites of a single immunoglobulin G *(IgG)* molecule can bind, leading to a higher-avidity bivalent interaction. The hinge region of the IgG molecule accommodates the shape change

needed for simultaneous engagement of both binding sites. *IgM* molecules have 10 identical antigen-binding sites that can theoretically bind simultaneously with 10 repeating determinants on a cell surface, resulting in a polyvalent, high-avidity interaction.

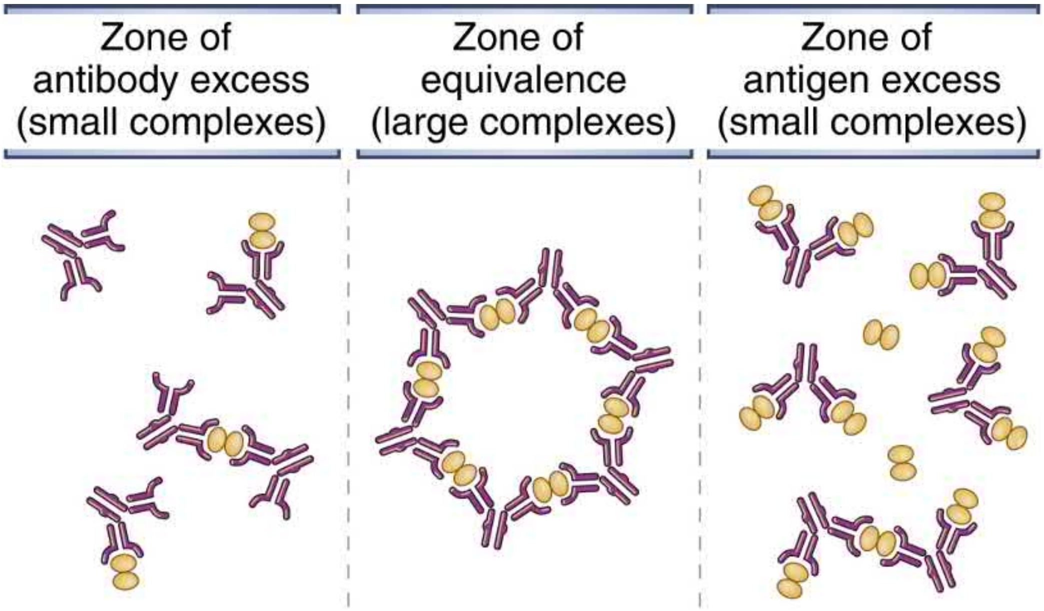
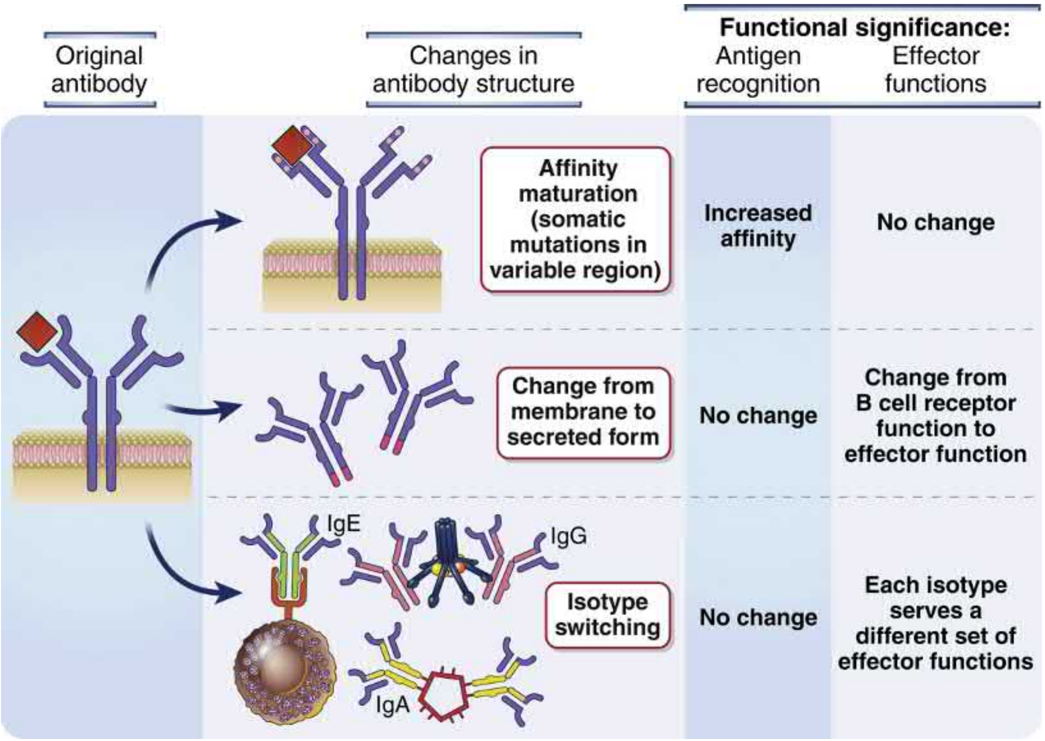

Zone of antibody excess (small complexes)
Zone of equivalence (large complexes)
Zone of antigen excess (small complexes)

FIGURE 5.15 Antigen-antibody complexes.The sizes of antigen-antibody (immune) complexes are a function of the relative concentrations of antigen and antibody. Large complexes are formed at concentrations of multivalent antigens and antibodies that are termed the zone of equivalence; the complexes are smaller in relative antigen or antibody excess.



Original antibody
Changes in antibody structure
Functional significance:
Antigen recognition
Effector functions
Affinity maturation (somatic mutations in variable region)
Increased affinity
No change
Change from membrane to secreted form
No change
Change from B cell receptor function to effector function
IgE
IgG
Isotype switching
IgA
No change
Each isotype serves a different set of effector functions

FIGURE 5.16 Changes in antibody structure during humoral immune responses.The illustration depicts the changes in the structure of antibodies that may be produced by the progeny of activated B cells (one clone) and the related changes in function. During affinity maturation, mutations in the V region (indicated by *yellow dots*) lead to changes in fine specificity without changes in C region–dependent effector functions. Activated B cells may shift production from largely membrane-bound antibodies containing transmembrane and cytoplasmic regions to secreted antibodies. Secreted antibodies may or may not show V gene mutations (i.e., secretion of antibodies occurs before and after affinity maturation). In isotype switching, the C regions change (indicated by color change from *purple* to *green, yellow,* or *pink*) without changes in the antigen-binding V region. Isotype switching is seen in membrane-bound and secreted antibodies. We will discuss

the molecular basis for these changes in Chapter 12.

Affinity Maturation

The ability of antibodies to neutralize toxins and infectious microbes is dependent on tight binding of the antibodies. As we have discussed, tight binding is achieved by high-affinity and high-avidity interactions. A mechanism for the generation of high-affinity antibodies involves subtle changes in the structure of the V regions of antibodies during humoral immune responses to protein antigens. These changes come about by a process of somatic mutation in antigen-stimulated B lymphocytes that generates new V domain structures, some of which bind the antigen with greater affinity than the original V domains (Fig. 5.16). Those B cells producing higher-affinity antibodies preferentially bind to the antigen and, as a result of selection, become the dominant B cells with each subsequent exposure to the antigen. This process, called **affinity maturation,** results in an increase in the average binding affinity of antibodies for an antigen as a humoral immune response evolves. Thus, an antibody produced during a primary immune response to a protein antigen often has a K_d in the range of 10^{-7} to 10^{-9} M; in subsequent responses to the same antigen (secondary responses), as may occur in repeated infections with the same species of microbe or repeated immunizations, the affinity increases, often resulting in a K_d of 10^{-11} M or even less. We will discuss the mechanism of affinity maturation in Chapter 12.

Features Related to Effector Functions

Many of the effector functions of antibodies are mediated by the Fc portions of the molecules, and Ig isotypes that differ in these Fc regions perform distinct functions. We have mentioned previously that the effector functions of antibodies require the binding of heavy chain C regions, which make up the Fc portions, to other cells and plasma proteins. For example, IgG coats microbes and targets them for phagocytosis by neutrophils and macrophages. This occurs because the IgG molecule is able to simultaneously bind through its Fab region to the microbe, and through its Fc region to IgG heavy chain–specific Fc receptors that are expressed on neutrophils and macrophages. In contrast, IgE binds to mast cells and triggers their degranulation because mast cells express IgE-specific Fc receptors. Another Fc-dependent effector mechanism of humoral immunity is activation of the classical pathway of the complement system. The system generates inflammatory mediators and promotes microbial phagocytosis and lysis. It is initiated by the binding of a complement protein called C1q to the Fc portions of antigen-complexed IgG or IgM. The Fc receptor–binding and complement-binding sites of antibodies are found within the heavy chain C domains of the different isotypes (see Fig. 5.1). We will discuss the structure and functions of Fc receptors and complement proteins in Chapter 13.

The effector functions of antibodies are initiated only by Ig molecules that have bound antigens and not by free Ig. The reason that only antibodies with bound antigens activate effector mechanisms is that two or more adjacent antibody Fc portions are needed to bind to and trigger various effector systems, such as complement proteins and Fc receptors of phagocytes (see Chapter 13). This requirement for adjacent antibody molecules ensures that the effector functions are targeted specifically toward eliminating antigens that are recognized by the antibody and that circulating free antibodies do not, inappropriately and dangerously, trigger effector responses.

Changes in the isotypes of antibodies during humoral immune responses influence how the responses work to eradicate antigens. After stimulation by an antigen, a single clone of B cells may produce antibodies with different isotypes that nevertheless possess identical V domains and therefore identical antigen specificity. Naive B cells simultaneously produce IgM and IgD that function as membrane receptors for antigens. When these B cells are activated by foreign antigens, typically of microbial origin, they may undergo a process called **isotype** (or **class**) **switching,** in which the type of C_H region, and therefore the antibody isotype, produced by the B cell changes, but the V regions and the specificity do not (see Fig. 5.16). As a result of isotype switching, different progeny of the original IgM- and IgD-expressing naive B cell may produce isotypes and subtypes that are best able to eliminate the antigen. For example, the antibody response to many bacteria and viruses in the blood is dominated by IgG antibodies, but the same microbes in mucosal tissues (intestines and airways) elicit much more IgA, which is efficiently secreted into the lumens of these organs. Switching to the IgG isotype also prolongs the effectiveness of humoral immune responses because of the long half-life of IgG antibodies. We will discuss the mechanisms and functional significance of isotype switching in Chapter 12.

The heavy chain C regions of antibodies also determine the tissue distribution of antibody molecules. As we mentioned earlier, after B cells are activated, they gradually lose expression of the membrane-bound antibody and express more of it as a secreted protein (see Fig. 5.16). IgA can be secreted efficiently across mucosal epithelia and is the major class of antibody in mucosal secretions and milk (see Chapter 14). Neonates are protected from infections by IgG antibodies they acquire from their mothers through the placenta during gestation. This transfer of maternal IgG is mediated by the FcRn, which we described earlier as the receptor responsible for the long half-life of IgG antibodies.

Summary

- Antibodies, or immunoglobulins (Igs), are a family of glycoproteins produced in membrane-bound or secreted form by B lymphocytes.
- Membrane-bound antibodies serve as receptors that mediate the antigen-triggered activation of B cells.
- Secreted antibodies function as mediators of specific humoral immunity by neutralizing microbes and toxins and

by engaging various effector mechanisms that serve to eliminate the bound antigens.

- The antigen-binding regions of antibody molecules are highly variable, and any one individual has the potential to produce millions of different antibodies, each with distinct antigen specificity.
- All antibodies have a common symmetric core structure of two identical covalently linked heavy chains and two identical light chains, each linked to one of the heavy chains. Each chain consists of two or more independently folded Ig domains of about 110 amino acids containing conserved sequences and intrachain disulfide bonds.
- The *N*-terminal domains of heavy and light chains form the V regions of antibody molecules, which differ among antibodies of different specificities. The V regions of heavy and light chains each contain three separate hypervariable regions of about 10 amino acids that are spatially assembled to form the antigen-combining site of the antibody molecule.
- Antibodies are classified into different isotypes and subtypes on the basis of differences in the heavy chain C regions, which consist of three or four Ig C domains, and these classes and subclasses have different functional properties. The antibody classes are called IgM, IgD, IgG, IgE, and IgA. Both light chains of a single Ig molecule are of the same isotype, either κ or λ, which differ in their single C domains.
- Most of the effector functions of antibodies are mediated by the C regions of the heavy chains, but these functions are triggered by binding of antigens to the combining site in the V region.
- Monoclonal antibodies are produced from a single clone of B cells and recognize a single antigenic determinant. Monoclonal antibodies can be generated in the laboratory and are widely used in research, diagnosis, and therapy.

- Antigens are substances specifically bound by antibodies or T lymphocyte antigen receptors. Antigens that bind to antibodies include a wide variety of biologic molecules, including sugars, lipids, carbohydrates, proteins, and nucleic acids. This is in contrast to most T cell antigen receptors, which recognize only peptide antigens.
- Macromolecular antigens contain multiple epitopes, or determinants, each of which may be recognized by an antibody. Linear epitopes of protein antigens consist of a sequence of adjacent amino acids, and conformational determinants are formed by folding of a polypeptide chain.
- The affinity of the interaction between the combining site of a single antibody molecule and a single epitope is generally represented by the K_d calculated from binding data.

  Polyvalent antigens contain multiple identical epitopes to which identical antibody molecules can bind. Antibodies can bind to 2 or, in the case of IgM, up to 10 identical epitopes simultaneously, leading to enhanced avidity of the antibody-antigen interaction.
- The relative concentrations of polyvalent antigens and antibodies may favor the formation of immune complexes that may deposit in tissues and cause damage.
- Antibody binding to antigen can be highly specific, distinguishing small differences in chemical structures, but cross-reactions may also occur in which two or more antigens may be bound by the same antibody.
- Several changes in the structure of antibodies made by one clone of B cells may occur in the course of an immune response. B cells initially produce only membrane-bound Ig, but in activated B cells and plasma cells, Ig with the same antigen-binding specificity as the original membrane-bound Ig receptor is secreted. Changes in the use of C region gene segments without changes in V regions are the basis of isotype switching, which leads to changes in effector function without a change in specificity. Point

mutations in the V regions of an antibody specific for an antigen lead to increased affinity for that antigen (affinity maturation).

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

Antibody Structure

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See https://www.nobelprize.org/prizes/medicine/1972/edelman/lecture.)

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Ehrlich P. Experimental investigations on immunity II. On Abrin. *Deutsch med Wochenschr.* 1891;17:1218–1219.

∗Porter RR. The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. *Biochem J* . 1959;73:119–126; and

Porter RR. Separation and isolation of fractions of rabbit gamma-globulin containing the antibody and antigenic combining sites. *Nature* . 1958;182:670–671. *(These studies on the identification of the Fab and Fc fragments of antibodies led to a Nobel prize for Rodney Porter. See* https://www.nobelprize.org/prizes/medicine/1972/porter/lecture.)

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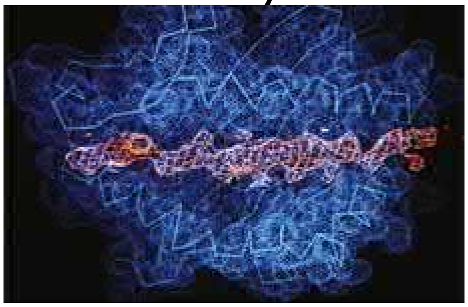
Chapter 6: Antigen Presentation to T Lymphocytes and the Function of Major Histocompatibility Complex Molecules



Properties of Antigens Recognized by T Lymphocytes,

Antigen Capture and the Functions of Antigen-Presenting Cells,
  - General Properties of Antigen-Presenting Cells,
  - Role of Dendritic Cells in Antigen Capture and Display,
  - Functions of Other Antigen-Presenting Cells,

The Major Histocompatibility Complex,
  - Discovery of the MHC,
  - MHC Genes,
  - Structure of MHC Molecules,
  - Binding of Peptides to MHC Molecules,

- Processing of Protein Antigens,
  - The Class I MHC Pathway for Processing and Presentation of Cytosolic Proteins,
  - The Class II MHC Pathway for Presentation of Proteins Degraded in Acidic Vesicles,
  - Physiologic Significance of MHC–Associated Antigen Presentation,
- Presentation of Nonprotein Antigens to T Cells,
- Summary,

The principal functions of T lymphocytes are to eradicate infections by intracellular microbes and to activate other cells, such as macrophages and B lymphocytes. The activation and functions of T cells have several features that reflect the special properties of this cell type.

To initiate immune responses, antigens are captured from their site of entry and concentrated in secondary (peripheral) lymphoid organs through which naive T cells circulate constantly. Microbes and other antigens most often enter the body through epithelium-lined surfaces, which interface with the external environment. Microbes may also colonize any tissue, and antigens may be produced in these tissues. Because the the total number of lymphocytes in the body is finite and the immune system generates a large number of lymphocyte clones each with a different specificity, there are very few naive T and B cells specific for any one antigen, in the range of 1 in 10^5 or 10^6 lymphocytes. This small number of naive T cells has to be able to locate and respond to the foreign antigen. It is impossible for the few T cells specific for any antigen to constantly patrol all the possible tissues where antigens may enter or be produced. The mechanism that solves this problem is a specialized system for capturing an antigen from its site of entry or production and bringing it to secondary lymphoid organs through which naive

T cells circulate. The cells that capture antigens and display them to T lymphocytes are called **antigen-presenting cells (APCs).** Once helper and cytotoxic effector T cells are produced, they leave the lymphoid organs and migrate to sites of infection and then recognize the same antigens that initiated the response again presented by cells at these sites. Some helper T cells migrate toward follicles and then recognize the same antigens presented by B cells. This second round of antigen presentation activates the effector functions of the T cells so that they can eliminate the microbes or activate the B cells.

T lymphocytes recognize and respond to cell-associated antigens and not to soluble, cell-free antigens. A principal function of T lymphocytes is to eliminate microbes that survive inside cells. In addition, T cells help B cells produce antibodies that kill microbes outside of cells. In both cases, the T cells must interact with and activate other cells, such as macrophages and B lymphocytes. Other T cells kill virus-infected cells and tumors. T cell antigen receptors have evolved to see antigens that are derived from proteins that are inside cells and are displayed by cell surface molecules, which ensures that T cells recognize cell-associated and not free antigens and interact with other cells. This is in striking contrast to B lymphocytes, whose antigen receptors and secreted products, antibodies, can recognize intact antigens on microbial and host cell surfaces, and soluble cell-free antigens. The task of displaying host cell–associated antigens for recognition by $CD4^+$ and $CD8^+$ T cells is performed by specialized proteins called **major histocompatibility complex (MHC)** molecules, which are expressed on the surfaces of host cells.

TABLE 6.1

Features of Major Histocompatibility Complex–Dependent Antigen Recognition by T Lymphocytes

Features of Antigens Recognized by T Cells	Explanation
Most T cells recognize peptides and no other molecules.	Only peptides bind to MHC molecules.
T cells recognize linear peptides and not conformational determinants of protein antigens.	Linear peptides bind to clefts of MHC molecules, and protein conformation is lost during the generation of these peptides.
T cells recognize cell-associated and not soluble antigens.	Most T cell receptors recognize only peptide-MHC complexes, and MHC molecules are membrane proteins that display stably bound peptides on cell surfaces.
CD4$^+$ and CD8$^+$ T cells preferentially recognize antigens ingested from the extracellular environment into vesicles and antigens present in the cytosol, respectively.	Pathways of assembly of MHC molecules ensure that class II MHC molecules display peptides that are proteolytically degraded in vesicles in APCs and class I MHC molecules present peptides from cytosolic proteins that are degraded by cytosolic proteasomes.

APCs, Antigen-presenting cells; *MHC*, major histocompatibility complex.

MHC molecules display antigens from different cellular compartments to different classes of T cells, such that the correct type of T cell recognizes the type of microbe that T cell is best at eliminating. For instance, defense against microbes in the circulation has to be mediated by antibodies, and the production of the most effective antibodies requires the participation of $CD4^+$ helper T cells. But if the same microbe (e.g., a virus) infects a tissue cell, it becomes inaccessible to the antibody, and its eradication may require that $CD8^+$ cytotoxic T lymphocytes (CTLs) kill the infected cells and eliminate the reservoir of infection. MHC molecules play a critical role in displaying antigens that are internalized from outside cells to $CD4^+$ T lymphocytes and those that are produced inside cells to $CD8^+$ T cells.

Elucidation of the cell biology and molecular basis of antigen presentation has been an impressive accomplishment, based on functional experiments, biochemical analyses, and structural biology. In this chapter, we will describe how antigens are captured and displayed to T cells. In Chapter 7, we will describe the antigen receptors of T cells, and in Chapters 9, 10, and 11, we will discuss the activation and effector functions of T lymphocytes.

Properties of Antigens Recognized by T Lymphocytes

Research on the nature of T cell antigen recognition showed as early as the 1960s that the physicochemical forms of antigens that are recognized by T cells are different from those recognized by B lymphocytes and antibodies. This knowledge led to the discovery of how antigens are seen by T cells. Several features of antigen recognition are unique to T lymphocytes (Table 6.1).

Most T lymphocytes recognize only short peptides, whereas B cells can recognize peptides, intact folded proteins, nucleic acids, carbohydrates, lipids, and small chemicals. As a result, T cell–mediated immune responses are usually induced by foreign protein antigens (the natural source of foreign peptides), whereas humoral immune responses are induced by protein and nonprotein antigens. Some T cells are specific for small chemical substances such as urushiol of poison ivy, β-lactams of penicillin antibiotics, and even metal ions such as nickel and beryllium. In these situations, it is likely that the chemicals bind to self proteins, including MHC molecules, and that T cells recognize the modified self peptides or altered MHC molecules. The peptide specificity of T cells is true for $CD4^+$ and $CD8^+$ cells; as we will discuss at the end of this chapter, there are some other, small populations of T cells that are capable of recognizing nonprotein antigens.

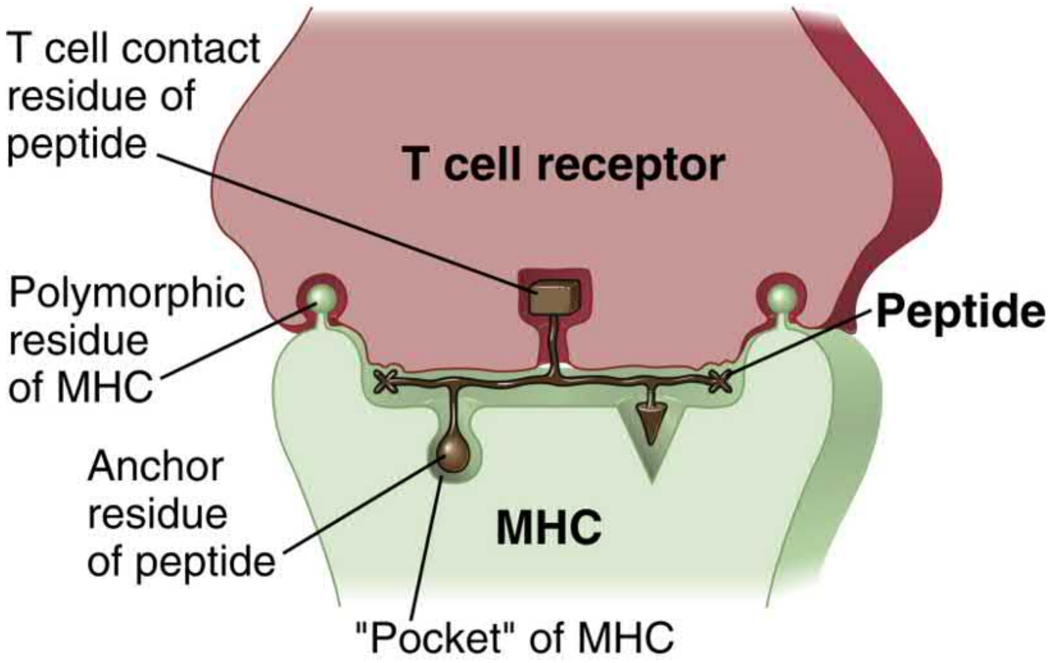

T cell contact residue of peptide
T cell receptor
Polymorphic residue of MHC
Peptide
Anchor residue of peptide
MHC
"Pocket" of MHC

FIGURE 6.1 A model for T cell recognition of a peptide-major histocompatibility complex. This schematic illustration shows an MHC molecule binding and displaying a peptide and a T cell receptor recognizing the complex of peptide and MHC molecule. As discussed later in the text, MHC-associated peptides contain some residues that anchor them into pockets in the cleft of the MHC molecule and other residues that are recognized by T cell antigen receptors. MHC residues that may vary among individuals (polymorphic residues) are also recognized by the T cell receptor. Thus, T cells see both peptide antigens and MHC molecules.

The antigen receptors of CD4$^+$ and CD8$^+$ T cells are specific for peptide antigens that are displayed by MHC molecules (Fig. 6.1) . The function of MHC molecules is to bind and display peptides for recognition by CD4$^+$ and CD8$^+$ T cells. As we will see in Chapter 8, MHC recognition is also required for the maturation of these T cells, ensuring that mature T cells are restricted to recognizing only

MHC molecules with bound antigens. MHC molecules can bind and display peptides and no other types of molecules; this is why CD4$^+$ and CD8$^+$ T cells recognize peptides. MHC molecules are highly polymorphic, and variations in MHC molecules among individuals influence both peptide binding and T cell recognition. A single T cell can recognize a specific peptide displayed by only one of the large number of different MHC molecules that exist. This phenomenon is called **MHC restriction,** and we will describe its molecular basis later in this chapter. There are two classes of MHC molecules, called class I and class II. CD4$^+$ T cells recognize peptides displayed by class II MHC, and CD8$^+$ T cells recognize peptides displayed by class I. The underlying mechanisms and functional importance of this separation are discussed later.

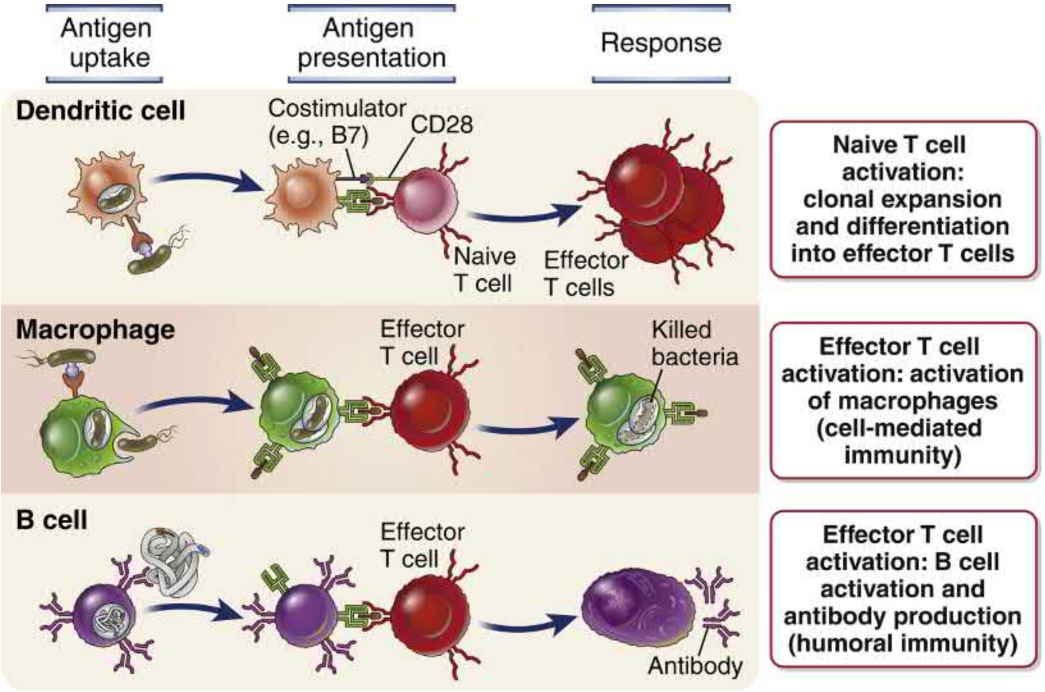

Antigen uptake
Antigen presentation
Response
Dendritic cell
Costimulator (e.g., B7)
CD28
Naive T cell
Effector T cells
Naive T cell activation: clonal expansion and differentiation into effector T cells
Macrophage
Effector T cell
Killed bacteria
Effector T cell activation: activation of macrophages (cell-mediated immunity)
B cell
Effector T cell
Antibody
Effector T cell activation: B cell activation and antibody production (humoral immunity)

FIGURE 6.2 Functions of different antigen-presenting cells. The three major types of antigen-resenting cells for CD4$^+$ T cells function to display antigens at different stages and in different types of immune responses. Note that effector T cells activate macrophages and B lymphocytes by production of cytokines and by expressing surface molecules; these will be described in later chapters.

We will start our discussion of antigen presentation by describing how APCs capture antigens and transport them to T cells.

Antigen Capture and the Functions of Antigen-Presenting Cells

The realization that various cells other than T cells are needed to present antigens to T lymphocytes first came from studies in which protein antigens that were known to elicit T cell responses were labeled and injected into mice, to determine which cells bound (and, by implication, recognized) these antigens. The result was that the injected antigens were associated mainly with nonlymphoid cells, which was a surprise because it was known that lymphocytes were the cells that specifically recognized and responded to foreign antigens. This type of experiment was quickly followed by studies showing that protein antigens that were physically associated with macrophages were much more immunogenic, on a molar basis, than the same antigens injected into mice in soluble form. In these early experiments, the macrophage populations studied may have contained dendritic cells (DCs), because, as we will discuss in the following section, naive T cells are best activated by antigens presented by DCs. Subsequent cell culture experiments showed that purified $CD4^+$ T cells could not respond to protein antigens, but they responded very well if non-T cells such as DCs or macrophages were added to the cultures. These results led to the concept that a critical step in the induction of a T cell response is the presentation of the antigen to T lymphocytes by other cells, which were named antigen-presenting cells (APCs). The first APCs identified were macrophages, and the responding T cells were $CD4^+$ helper cells. It soon became clear that several cell populations can function as APCs in different situations. By convention, APC is still the term used to refer to specialized cells that display antigens to $CD4^+$ T lymphocytes. As we will see later in this chapter, all nucleated cells can display peptide antigens to $CD8^+$ T lymphocytes, but they are not all called APCs.

General Properties of Antigen-Presenting Cells

Different cell types function as APCs to activate naive T cells or previously differentiated effector T cells (Fig. 6.2 and Table 6.2) **.** DCs are the most effective APCs for activating naive T cells and therefore for initiating T cell responses. Macrophages and B lymphocytes also function as APCs, but mostly for previously activated CD4$^+$ helper T cells rather than for naive T cells. Their roles as APCs are described later in this chapter and in more detail in Chapters 10 and 12. DCs, macrophages, and B lymphocytes express class II MHC molecules and are therefore capable of activating CD4$^+$ T lymphocytes. For this reason, these three cell types have been called professional APCs; however, this term is sometimes used to refer only to DCs because of their unique role in naive T cell activation.

TABLE 6.2

	Expression of		
Cell Type	**Class II Major Histocompatibility Complex**	**Costimulators**	**Principal Function**
Dendritic cells	Constitutive; increases with maturation; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Constitutive; expression is increased with TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to naive T cells in initiation of T cell responses to protein antigens (priming)
Macrophages	Low or negative; increased by IFN-γ and T cells (CD40L-CD40 interactions)	Expression is increased by TLR signals, IFN-γ, CD40-CD40L interactions	Antigen presentation to effector CD4$^+$ T cells in effector phase of cell-mediated immune responses (T cell–enhanced killing of phagocytosed microbes)
B lymphocytes	Constitutive; increased by IL-4, antigen receptor cross-linking, and T cells (CD40L-CD40 interactions)	Expression is increased by T cells (CD40-CD40L interactions), antigen receptor cross-linking	Antigen presentation to CD4$^+$ helper T cells in humoral immune responses (helper T cell–B cell interactions)
Vascular endothelial cells	Inducible by IFN-γ; constitutive in some human blood vessels	Low; may be inducible	May promote activation of antigen-specific T cells at site of antigen exposure and in organ grafts
Thymic epithelial cells	Constitutive	Probably none	Positive and negative selection of developing CD4$^+$ T cells
Various epithelial and mesenchymal cells	Inducible by IFN-γ	Probably none	No known physiologic function; possible role in inflammatory diseases

IFN-γ, Interferon-γ; *IL-4,* interleukin-4; *LPS,* lipopolysaccharide; *MHC,* major histocompatibility complex.

[a] As mentioned in the text, class I MHC molecules are expressed on all nucleated cells and display peptides to CD8$^+$ T lymphocytes.

APCs display peptide-MHC complexes for recognition by T cells and also provide additional stimuli that are required for the full responses of the T cells. Antigen is the first signal, and these additional stimuli are sometimes called second signals. They are more important for activation of naive T cells than for restimulation of previously activated effector and memory cells. The membrane-bound molecules of APCs that function together with antigens to stimulate T cells are called **costimulators.** APCs also secrete cytokines that play critical roles in the differentiation of naive T cells into effector cells. These costimulators and cytokines are described in Chapters 9 and 10.

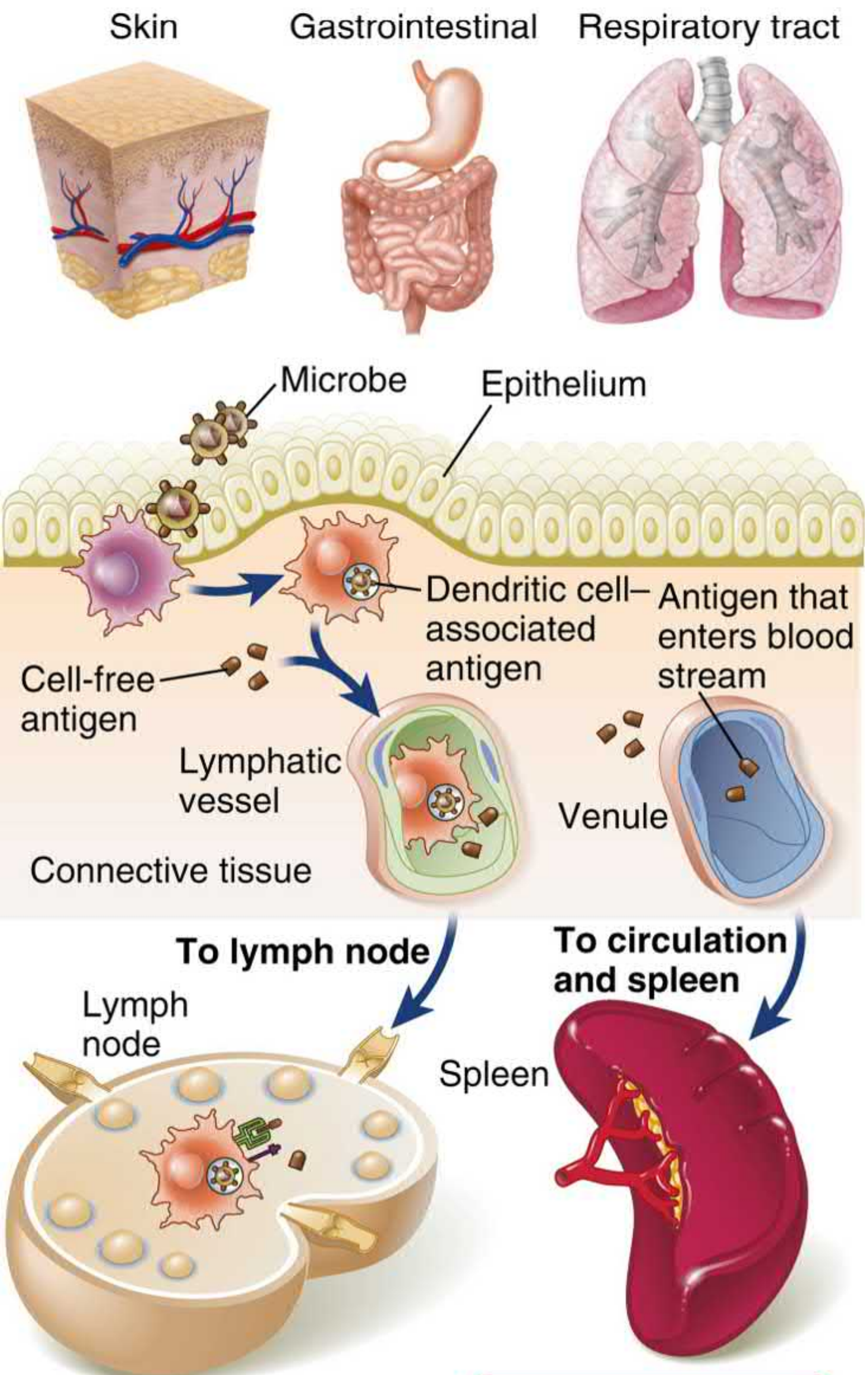
The antigen-presenting function of APCs is enhanced by exposure to microbial products. This is one reason that the immune system responds better to microbes than to harmless, nonmicrobial substances. DCs and macrophages express Toll-like receptors and other innate immune microbial sensors (see Chapter 4) and respond to microbes by increasing the expression of MHC molecules and costimulators, by improving the efficiency of antigen presentation, and by activating the APCs to produce cytokines, all of which help stimulate T cell responses. In addition, DCs that are activated by microbes express chemokine receptors that facilitate their migration to sites where naive T cells are present. The induction of optimal T cell responses to purified protein antigens in the absence of infection requires that the antigens be administered with substances called **adjuvants.** Adjuvants either are products of microbes, such as killed mycobacteria (used experimentally), or substances that elicit innate immune responses, like microbes do, and thus enhance the expression of costimulators and cytokines and also stimulate the antigen-presenting functions of APCs. Adjuvants are routinely used in animal studies of immune responses and in human vaccines.

APCs that present antigens to T cells also receive signals back from these lymphocytes that enhance the antigen-presenting function of the APCs. In particular, $CD4^+$ T cells that are activated by antigen recognition and costimulation express surface molecules, notably one called CD40 ligand (CD154), which binds to CD40 on DCs and macrophages, and the T cells also secrete cytokines, such as interferon-γ (IFN-γ), that bind to their receptors on these APCs. The combination of CD40 signals and cytokines activates the APCs, resulting in increased ability to process and present antigens, increased expression of costimulators, and secretion of cytokines that activate the T cells. This bidirectional interaction between

APCs displaying the antigen and T lymphocytes that recognize the antigen functions as a positive feedback loop that plays an important role in maximizing the immune response (see Chapter 9).

Role of Dendritic Cells in Antigen Capture and Display

Microbes and protein antigens that enter through epithelia are concentrated in lymph nodes, and blood-borne antigens are captured mostly in the spleen (Fig. 6.3) **.** The common routes through which foreign antigens, such as microbes, enter a host are the skin and the epithelia of the gastrointestinal and respiratory systems. In addition, microbial antigens may be produced in any tissue that has been colonized or infected by a microbe. The skin, mucosal epithelia, and parenchymal organs contain numerous lymphatic capillaries that drain lymph from these sites and into the regional lymph nodes. Some antigens are transported in the lymph by APCs (primarily DCs) that capture the antigen and enter lymphatic vessels, and other antigens enter the lymphatics in cell-free form. Thus, the lymph contains a sampling of all the soluble and cell-associated antigens that enter through epithelia and are present in tissues. The antigens become concentrated in lymph nodes, which are interposed along lymphatic vessels and act as filters that sample the lymph before it reaches the blood (see Chapter 2). Antigens that enter the blood stream may be sampled by APCs that are resident in the spleen or captured by circulating DCs and taken to the spleen.


Skin
Gastrointestinal
Respiratory tract
Microbe
Epithelium
Dendritic cell–associated antigen
Antigen that enters blood stream
Cell-free antigen
Lymphatic vessel
Venule
Connective tissue
To lymph node
To circulation and spleen
Lymph node
Spleen

Lymph node collects antigen from epithella and connective tissue

Blood-borne antigens are captured by antigen presenting cells in the spleen

FIGURE 6.3 Routes of antigen entry.Microbial antigens commonly enter through the skin and gastrointestinal and respiratory tracts, where they are captured by dendritic cells and transported to regional lymph nodes. Antigens that enter the bloodstream are captured by antigen-presenting cells in the spleen.

DCs are the cells that are best able to capture and transport antigens for presentation to naive T cells. DCs were introduced in Chapter 2, and their functions as tissue-resident sentinels that recognize microbes and trigger innate immune reactions were discussed in Chapter 4. Here we describe the role of these cells in antigen presentation to T lymphocytes.

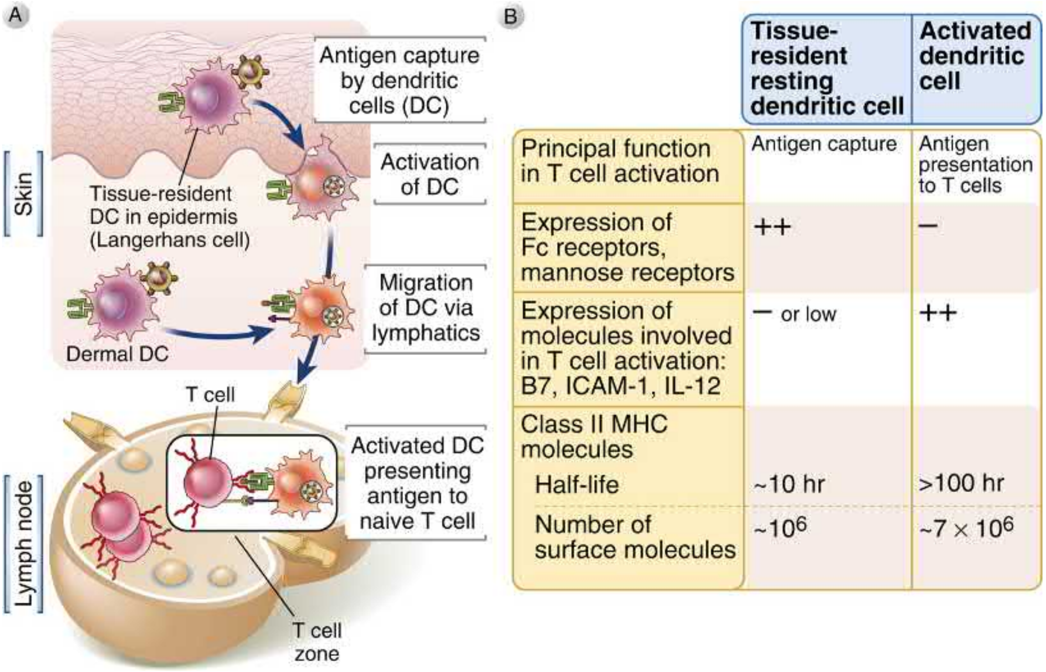
DCs are divided into several subsets based on phenotypes and functions.

- *Conventional (or classical) DCs (cDCs)* are present in most epithelia that interface with the external environment, such as the skin and the intestinal and respiratory tracts, and in tissues, and are enriched in lymphoid organs. They are the DC subset that captures antigens and transports them to secondary lymphoid organs and are thus involved in antigen presentation to naive $CD4^+$ and $CD8^+$ T cells. Conventional DCs are divided into two groups.
    - *Type 1 cDCs (cDC1)* are especially efficient at transferring ingested antigens from vesicles into the cytosol. As we will discuss later, this is an essential step in the process of cross-presentation, in which ingested antigens are presented on class I MHC molecules to $CD8^+$ T cells.
    - *Type 2 cDCs (cDC2)* are the major DC subset that presents captured antigens to $CD4^+$ T cells, and thus the subset that is most important for initiating responses of these T cells.

- *Plasmacytoid DCs (pDC)* are the body's major source of type I IFN and are thus essential for innate immune responses to viruses. pDCs also may capture antigens in the blood and transport them to the spleen.
- *Monocyte-derived DCs (moDC)* can be induced to develop from monocytes under inflammatory conditions. Their roles in immune responses are not clear.
- *Langerhans cells* of the epidermis were one of the earliest DCs identified. These cells are related to tissue-resident macrophages and develop early in life from progenitors in the yolk sac or fetal liver and seed the skin. Their function is probably similar to that of cDC2.

DCs that are resident in epithelia and tissues capture protein antigens. Tissue-resident cDCs express numerous membrane receptors, such as C-type lectins, that bind microbes. DCs use these receptors to capture and endocytose microbes or microbial proteins and then process the ingested proteins into peptides capable of binding to MHC molecules. In addition to receptor-mediated endocytosis and phagocytosis, DCs can ingest antigens by pinocytosis, a process that does not involve specific recognition receptors but serves to internalize whatever molecules might be in the fluid phase in the vicinity of the DCs.

Simultaneously with antigen capture, DCs are activated by microbial products to mature into APCs that transport the captured antigens to draining lymph nodes (Fig. 6.4)**.** At the time that microbial antigens are being captured, microbial products (i.e., pathogen-associated molecular patterns [PAMPs]), different from the protein antigens that T cells recognize, are recognized by Toll-like receptors and other innate pattern recognition receptors in the DCs and other cells, generating innate immune responses (see Chapter 4). The DCs are activated by these signals and by cytokines, such as tumor necrosis factor (TNF), produced in response to the microbes. The activated DCs (also called mature DCs) lose their adhesiveness for epithelia or tissues and begin to express a chemokine receptor called CCR7 that is specific for two chemokines, CCL19 and CCL21, that are produced in lymphatic vessels and in the T cell zones of lymph nodes. These chemokines attract the DCs bearing microbial antigens into draining lymphatics and ultimately into the T cell zones of the regional lymph nodes. Naive T cells also express CCR7, and this is why they localize to the same regions of lymph nodes where antigen-bearing DCs are concentrated (see Chapter 3), although their route into the lymph node is via the blood. The colocalization of antigen-bearing activated DCs and naive T cells maximizes the chance of T cells with receptors for the antigen finding that antigen.


A
Skin
Antigen capture by dendritic cells (DC)
Activation of DC
Tissue-resident DC in epidermis (Langerhans cell)
Migration of DC via lymphatics
Dermal DC
T cell
Lymph node
Activated DC presenting antigen to naive T cell
T cell zone
B

	Tissue-resident resting dendritic cell	Activated dendritic cell
Principal function in T cell activation	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	–
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life	~10 hr	>100 hr
Number of surface molecules	~10^6	~7×10^6

FIGURE 6.4 Role of dendritic cells in antigen capture and presentation. **A,** Immature dendritic cells (DCs) in the skin (Langerhans cells) or dermis (dDCs) capture antigens that enter through the epidermis and transport the antigens to regional lymph nodes. During this migration, the dendritic cells mature and become efficient APCs. **B,** The table summarizes some of the changes during DC maturation that are important in the functions of these cells. Half-life is an estimate of how long the molecules are expressed on cells. The number of surface molecules is per class II--expressing cell. *ICAM-1,* Intercellular adhesion molecule 1; *IL-12,* interleukin-12; *MHC,* major histocompatibility complex.

Activation also converts the DCs from cells whose primary function is to capture antigen into cells that are able to present antigens to naive T cells and to activate the lymphocytes. Activated DCs express high levels of MHC molecules with bound peptides and costimulators required for T cell activation. Thus, by the time these cells arrive in the lymph nodes, they have developed into potent APCs with the ability to activate T lymphocytes. Naive T cells that recirculate through lymph nodes encounter these APCs, and the T cells that are specific for the displayed peptide-MHC complexes are activated. This is the initial step in the induction of T cell responses to protein antigens.

In the absence of infection or inflammation, conventional DCs capture antigens in the tissues but are not activated to produce the high levels of cytokines and costimulators that are required to induce effective immune responses. The function of these DCs may be to present self antigens to self-reactive T cells and thereby cause inactivation or death of the T cells or generate regulatory T cells. These mechanisms play a role in maintaining self-tolerance and preventing autoimmunity (see Chapter 15).

Antigens are also transported to lymphoid organs in soluble form. Resident DCs in the lymph nodes and spleen may capture lymph- and blood-borne antigens, respectively, and also may be driven to mature by microbial products. When lymph enters a lymph node through an afferent lymphatic vessel, it drains into the subcapsular sinus, and some of the lymph enters fibroblast reticular cell (FRC) conduits that originate from the sinus and traverse the cortex (see Chapter 2). Once in the conduits, low-molecular-weight antigens can be extracted by DCs that line the outside surfaces of the conduits and whose processes interdigitate between the FRCs. Other antigens in the subcapsular sinus are taken up by macrophages, which carry the antigens into follicles and present these antigens to resident B cells. B cells in the node may also recognize and internalize soluble antigens.

The collection and concentration of foreign antigens in lymph nodes are supplemented by other anatomic adaptations that serve similar functions. The mucosal surfaces of the GI and respiratory systems, in addition to being drained by lymphatic capillaries, contain specialized collections of secondary lymphoid tissue that can directly sample the luminal contents of these organs for the presence of antigenic material. The best characterized of these mucosal lymphoid organs are Peyer’s patches of the ileum and the pharyngeal tonsils (see Chapter 14). APCs in the spleen monitor the blood stream for any antigens that reach the circulation. Such antigens may reach the blood either directly from the tissues or by way of the lymph from the thoracic duct.

Several properties of conventional DCs make them the most efficient APCs for initiating primary T cell responses.

- DCs are strategically located at the common sites of entry of microbes and foreign antigens (in epithelia) and in tissues that may be colonized by microbes.
- DCs express receptors that enable them to capture and respond to microbes.
- In response to chemokines, activated DCs migrate from epithelia and tissues via lymphatics, preferentially into the T cell zones of lymph nodes, and naive T lymphocytes also circulate through the same regions of the lymph nodes.
- Mature DCs express high levels of peptide-MHC complexes, costimulators, and cytokines, all of which are needed to activate naive T lymphocytes.
- Specialized DCs (cDC1) can transfer internalized proteins from phagosomes into the cytosol and are thus efficient at cross-presenting antigens to $CD8^+$ T cells. As we will see later, this process is essential for initiating $CD8^+$ T cell responses to many viruses and tumors.

Functions of Other Antigen-Presenting Cells

Although DCs have a critical role in initiating primary T cell responses, other cell types are also important APCs in different situations (see Fig. 6.2 and Table 6.2).

In cell-mediated immune responses, macrophages present the antigens of phagocytosed microbes to effector T cells, which respond by activating the macrophages to kill the ingested microbes. This process is central to cell-mediated immunity (see Chapter 10). Circulating monocytes are able to migrate to any site of infection and inflammation, where they differentiate into macrophages and phagocytose microbes as a prelude to destruction. Tissue-resident macrophages serve the same functions. $CD4^+$ T cells recognize microbial antigens being presented by the macrophages and provide signals that enhance the microbicidal activities of these macrophages. The requirement for specific antigen recognition means that T cells activate only macrophages containing the microbe that is the source of the antigen.

In humoral immune responses, B lymphocytes internalize protein antigens and present peptides derived from these proteins to helper T cells. This antigen-presenting function of B cells is essential for helper T cell–dependent antibody production and for maintaining specificity in humoral immune responses (see Chapter 12).

All nucleated cells can present peptides, derived from cytosolic protein antigens, to $CD8^+$ CTLs. All nucleated cells are susceptible to viral infections and cancer-causing mutations. Therefore, it is important that the immune system be able to recognize cytosolic antigens, such as viral antigens and mutated proteins, in any cell type. $CD8^+$ CTLs are the cell population that recognizes these antigens and eliminates the cells in which the antigens are produced. $CD8^+$ CTLs may also recognize phagocytosed microbes if these microbes or their antigens escape from phagocytic vesicles into the cytosol.

Other cell types that express class II MHC molecules and may present antigens to T cells include endothelial and some epithelial cells. Vascular endothelial cells may present antigens to blood T cells that adhere to vessel walls, but the role of this process in cell-mediated immune reactions is unclear. Endothelial cells in grafts also are targets of T cells reacting against graft antigens (see Chapter 17). Various epithelial and mesenchymal cells may express class II MHC molecules in response to the cytokine IFN-γ. The physiologic significance of antigen presentation by these cell populations is not established. Because most of them do not express costimulators and are not efficient at processing proteins into MHC-binding peptides, it is unlikely that they contribute significantly to the majority of T cell responses. Thymic epithelial cells constitutively express MHC molecules and play a critical role in presenting peptide-MHC complexes to maturing T cells in the thymus as part of the selection processes that shape the repertoire of T cell specificities (see Chapter 8).

Now that we have described the functions of APCs and how antigens are captured from the environment and taken to lymphoid organs, we turn to the mechanism of antigen display and especially the role of MHC molecules in this process.

The Major Histocompatibility Complex

The discovery of the role of the MHC in antigen recognition by $CD4^+$ and $CD8^+$ T cells has been fundamental to our current understanding of the activation and functions of lymphocytes. The MHC was discovered from studies of tissue transplantation in mice, and it was many years later that the structure and function of MHC molecules were elucidated.

Discovery of the MHC

The Mouse MHC (H-2 Complex)

It was known from the early days of transplantation that tissues, such as skin, exchanged between nonidentical individuals are rejected, whereas the same grafts between identical twins are accepted. This result showed that tissue rejection is a genetically determined process. Early in the 20th century, inbred mouse strains were created by repetitive mating of siblings. Inbred mice are homozygous at every genetic locus (i.e., they have two copies of the same allele of every gene, one from each parent), and every mouse of an inbred strain is genetically identical (syngeneic) to every other mouse of the same strain (i.e., they all express the same alleles). Different strains may express different alleles and are said to be allogeneic to one another. In the 1940s, George Snell and colleagues created substrains of inbred mice called congenic mice that were identical at all loci except for one on chromosome 17. This difference allowed one congenic strain with a specific allele on chromosome 17 to reject a graft from another strain that had a different allele at this locus, but was otherwise genetically identical. Because this allelic locus determined the compatibility of tissue grafts among different strains, it was called the major histocompatibility locus (*histo,* tissue). The particular locus that was identified in mice contained a gene encoding a blood group antigen called antigen II, and, therefore, this region was named histocompatibility-2, or simply H-2. Initially, this locus was thought to contain a single gene that controlled tissue compatibility. However, occasional recombination events occurred within the H-2 locus during interbreeding of different strains, indicating that it actually contained several different but closely linked genes, many of which were involved in graft rejection. The genetic region that controlled graft rejection and contained several linked genes was named the **major histocompatibility complex (MHC)**. Although not known at the time of the initial experiments, transplant rejection is in large part a T cell–mediated process (see Chapter 17), and, therefore, it is not surprising that there is a relationship between graft rejection and MHC genes, which encode the peptide-binding MHC molecules that T cells recognize.

The Human MHC (Human Leukocyte Antigen Locus)

The human MHC was discovered by searching for cell surface molecules in one individual that would be recognized as foreign by another individual. This task became feasible when it was discovered that individuals who had received multiple blood transfusions and patients who had received kidney transplants had antibodies that recognized cells from the blood or kidney donors, and that multiparous women had circulating antibodies that recognized paternal cells. The proteins recognized by these antibodies were called **human leukocyte antigens (HLAs)** (leukocyte because the antibodies were tested by binding to the leukocytes of other individuals, and antigens because the molecules were recognized by antibodies). Subsequent analyses showed that as in mice, the inheritance of genes (HLA alleles) encoding particular HLA antigens is a major determinant of graft acceptance or rejection (see Chapter 17). Biochemical studies gave the satisfying result that the proteins encoded in the mouse H-2 locus and the HLA proteins identified in humans had very similar basic structures. From these results came the conclusion that genes that determine the fate of grafted tissues are present in all mammalian species and are homologous to the H-2 genes first identified in mice; these are called MHC genes. Other polymorphic genes that contribute to graft rejection to a lesser degree are called minor histocompatibility genes; we will return to these in Chapter 17, when we discuss transplantation immunology.

Immune Response Genes

For almost 20 years after the MHC was discovered, its only documented role was in graft rejection. This was a puzzle to immunologists because transplantation is not a natural phenomenon and there was no reason that a set of genes should be preserved through evolution if the only function of the genes was to stimulate the rejection of foreign tissue grafts. In the 1960s and 1970s, it was discovered that MHC genes are of fundamental importance for all immune responses to protein antigens. Baruj Benacerraf, Hugh McDevitt, and colleagues found that inbred strains of a single species (guinea pigs or mice) differed in their ability to make antibodies against some simple synthetic polypeptides and responsiveness was inherited as a dominant Mendelian trait. The relevant genes were called immune response (Ir) genes, and they were all located in the MHC. We now know that Ir genes are, in fact, class II MHC genes that encode class II MHC molecules that differ in their ability to bind and display peptides derived from various protein antigens. Responder strains, which can mount immune responses to a particular polypeptide antigen, inherit MHC alleles whose products can bind peptides derived from these antigens, forming peptide-MHC complexes that can be recognized by helper T cells. These T cells then help B cells to produce antibodies. Nonresponder strains express MHC molecules that are not capable of binding peptides derived from the polypeptide antigen, and, therefore, these strains cannot generate helper T cells or antibodies specific for the antigen. It was also later found that many autoimmune diseases were associated with the inheritance of particular MHC alleles, firmly placing these genes at the center of the mechanisms that control immune responses. Such studies provided the impetus for more detailed analyses of MHC genes and proteins.

The Phenomenon of MHC Restriction

The formal proof that the MHC is involved in antigen recognition by T cells came from the experimental demonstration of MHC restriction by Rolf Zinkernagel and Peter Doherty. In their classic study, reported in 1974, these investigators examined the recognition of virus-infected cells by virus-specific CTLs in inbred mice. If a mouse is infected with a virus, $CD8^+$ T cells specific for the virus are activated and differentiate into CTLs in the animal. When the function of these CTLs is analyzed in vitro, they recognize and kill virus-infected cells only if the infected cells express MHC molecules that are expressed in the animal from which the CTLs were obtained (Fig. 6.5). Thus, T cells must be specific not only for the antigen but also for MHC molecules, and T cell antigen recognition is restricted by the MHC molecules a T cell sees. Subsequent studies established that the recognition of antigens by $CD8^+$ CTLs is restricted by class I MHC molecules, and the responses of $CD4^+$ helper T lymphocytes to antigens are restricted by class II MHC molecules.

We will continue our discussion of the MHC by describing the properties of the genes and then the proteins, and we will conclude by describing how these proteins bind and display foreign antigens.

MHC Genes

The MHC locus contains two types of polymorphic MHC genes, the class I and class II MHC genes, which encode two groups of structurally distinct but homologous proteins, and other nonpolymorphic genes whose products are involved in antigen presentation (Fig. 6.6) **.** Polymorphism refers to variations in a gene among individuals in an outbred population. The class I and class II MHC molecules are the ones whose function is to display peptide antigens for recognition by $CD8^+$ and $CD4^+$ T cells, respectively. The nonpolymorphic molecules encoded in the MHC do not present peptides for T cell recognition.

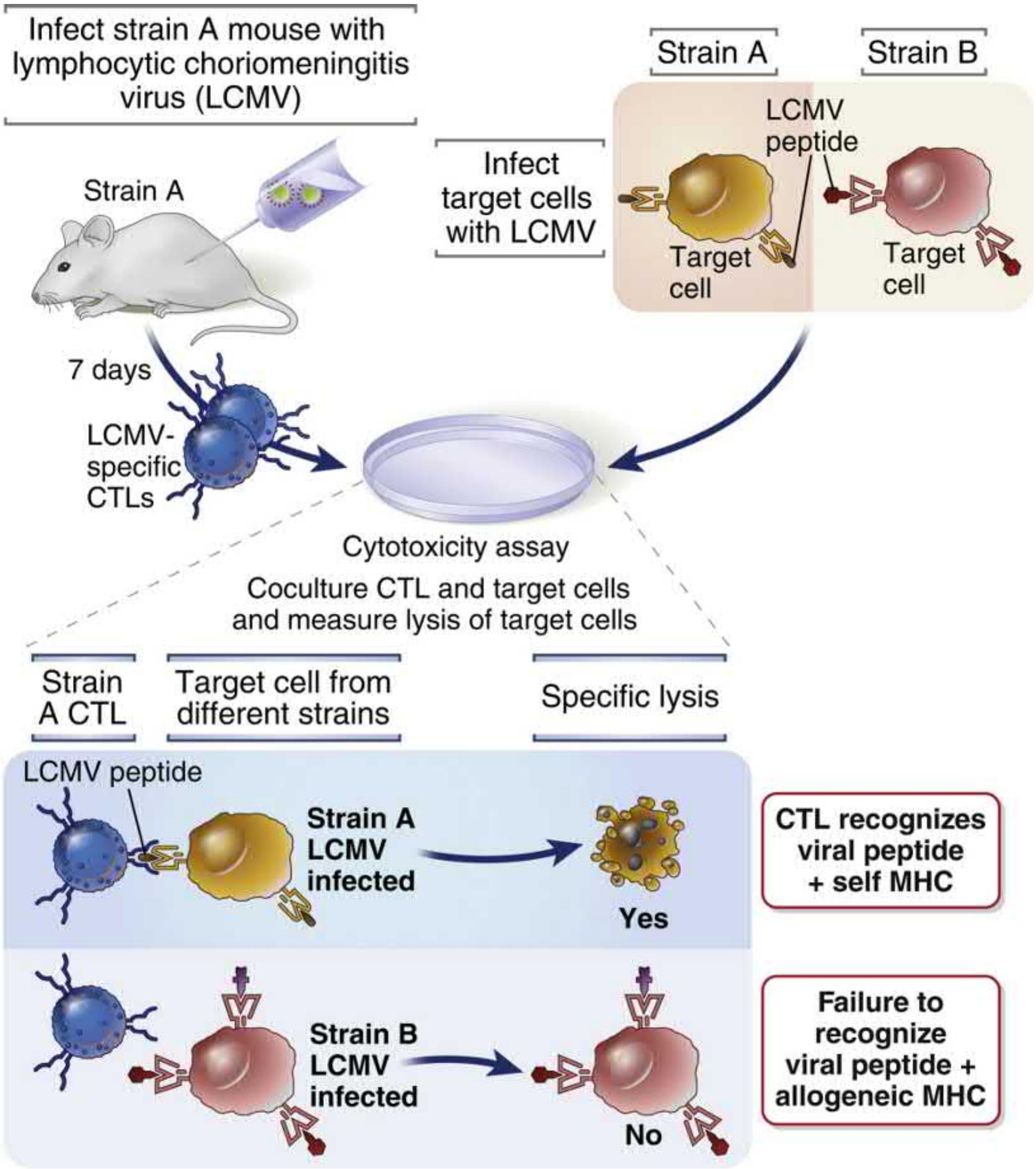

Infect strain A mouse with lymphocytic choriomeningitis virus (LCMV)
Strain A
7 days
LCMV-specific CTLs
Infect target cells with LCMV
Strain A
Strain B
LCMV peptide
Target cell
Target cell
Cytotoxicity assay
Coculture CTL and target cells and measure lysis of target cells
Strain A CTL
Target cell from different strains
Specific lysis
LCMV peptide
Strain A LCMV infected
Yes
CTL recognizes viral peptide + self MHC
Strain B LCMV infected
No
Failure to recognize viral peptide + allogeneic MHC

FIGURE 6.5 Experimental demonstration of the phenomenon of major histocompatibility complex *(MHC)* restriction of T lymphocytes. Virus-specific cytotoxic T lymphocytes *(CTLs)* generated from virus-infected strain A mice kill only syngeneic (strain A) target cells infected with that virus. The CTLs do not kill infected strain B targets (which express different MHC alleles than does strain A). By use of congenic mouse strains that differ only at class I MHC loci, it has been proved that recognition of antigen by CD8$^+$ CTLs is self class I

MHC restricted. *LCMV*, Lymphocytic choriomeningitis virus.

Different human class I HLA molecules were first distinguished by serologic approaches (antibody binding). Different class II MHC molecules were identified by use of assays in which T cells from one individual would be activated by cells of another individual (called the mixed lymphocyte reaction). Currently, DNA sequencing is used to distinguish different MHC alleles and their encoded proteins.

Class I and class II MHC genes are the most polymorphic genes present in any mammalian genome. A remarkable feature to emerge from the studies of human MHC genes is the unexpected extent of polymorphism. In the population, the total number of HLA alleles with different amino acid sequences is estimated to be over 14,000, with more than 3500 variants for the HLA-B locus alone. The variations in MHC molecules (accounting for the polymorphism) result from inheritance of distinct DNA sequences and are not induced by gene recombination (as they are in antigen receptors; see Chapter 8). Because the products of different MHC alleles bind and display different peptides, different individuals in a population may present different peptides even from the same protein antigen.

The high degree of polymorphism of the MHC helps provide protection of mammalian populations from a virtually unlimited diversity of microbes and therefore prevents loss of entire populations from emerging infections. In other words, because of the preservation of a large number of different MHC molecules in the population, there will almost always be individuals able to present peptides from almost any microbe to their T cells. The evolution of new MHC alleles is an ongoing process. It occurs by a mechanism called gene conversion, which involves the copying of nucleotide sequences from one allele to another during meiosis. The selective pressures that drive this process and have preserved such a vast number of alleles in the population are not understood.

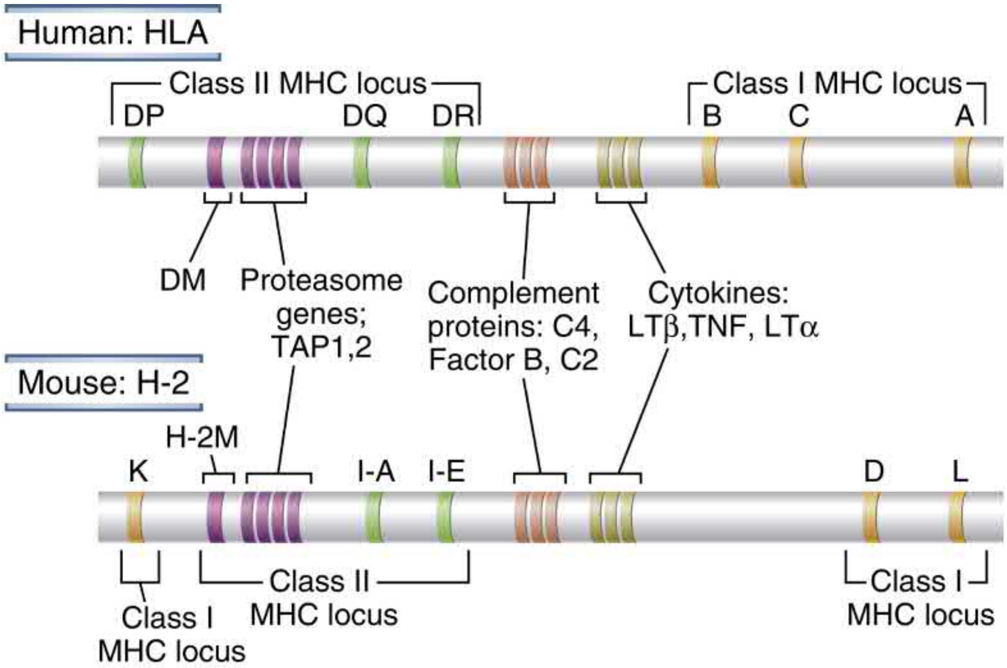

Human: HLA
Class II MHC locus
DP
DQ
DR
Class I MHC locus
B
C
A
DM
Proteasome genes; TAP1,2
Complement proteins: C4, Factor B, C2
Cytokines: LTβ,TNF, LTα
Mouse: H-2
H-2M
K
I-A
I-E
D
L
Class I MHC locus
Class II MHC locus
Class I MHC locus

FIGURE 6.6 Schematic maps of human and mouse major histocompatibility complex loci. The basic organization of the genes in the MHC locus is similar in humans and mice. Sizes of genes and intervening DNA segments are not shown to scale. Class II loci are shown as single blocks, but each locus consists of several genes. *HLA,* Human leukocyte antigen; *LT,* lymphotoxin; *TAP,* transporter associated with antigen processing; *TNF,* tumor necrosis factor.

MHC genes are codominantly expressed in each individual. In other words, for a given MHC gene, each individual expresses the alleles that are inherited from both parents. For the individual, this maximizes the number of MHC molecules available to bind peptides for presentation to T cells.

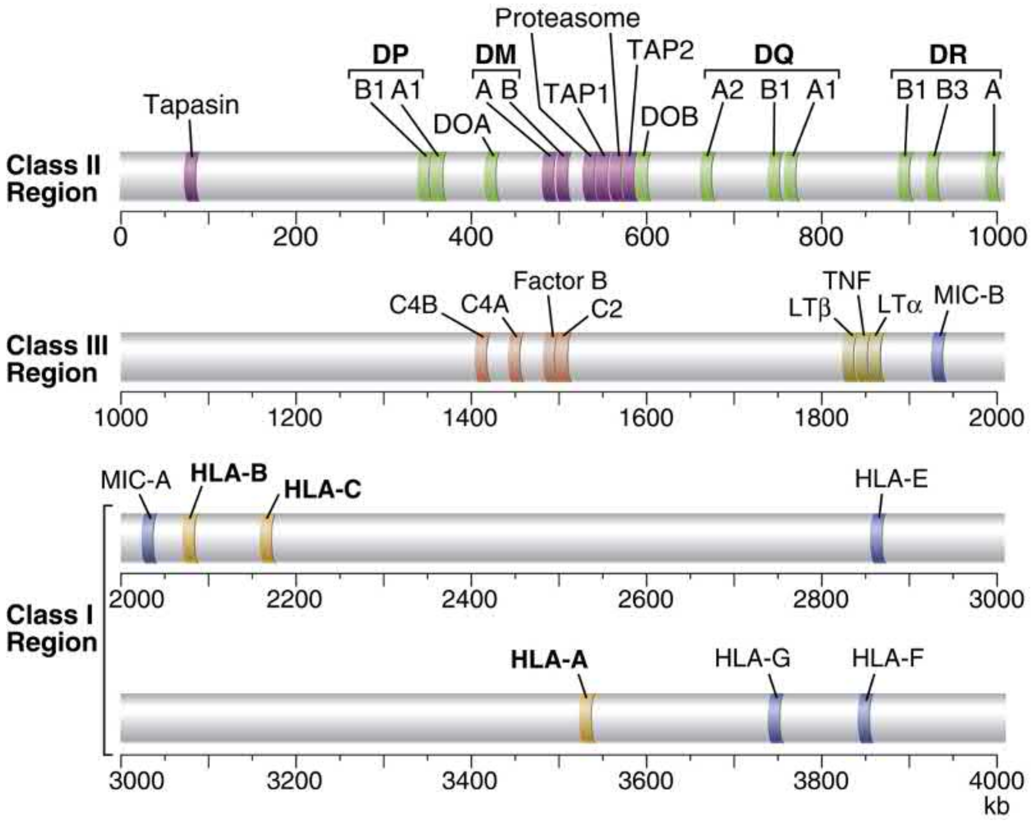
Human and Mouse MHC Gene Loci

In humans the MHC is located on the short arm of chromosome 6 and occupies a large segment of DNA, extending about 3500 kilobases (kb). In classical genetic terms, the MHC locus extends about 4 centimorgans, meaning that crossovers within the MHC occur in about 4% of meioses. A molecular map of the human MHC is shown in Fig. 6.7.

There are three class I MHC genes called *HLA-A, HLA-B,* and *HLA-C,* which encode three types of class I MHC molecules with the same names. There are three class II HLA gene loci called *HLA-DP, HLA-DQ,* and *HLA-DR*. Each class II MHC molecule is composed of a heterodimer of α and β polypeptides. The *DP, DQ,* and *DR* loci on each chromosome contain separate genes designated *A* and *B,* encoding the α and β chains, respectively. Every individual has two *HLA-DP* genes (called *DPA1* and *DPB1*), two *HLA-DQα* genes (*DQA1,* 2), one *HLA-DQβ* gene (*DQB1*), one *HLA-DRα* gene (*DRA1*), and one or two *HLA-DRβ* genes (*DRB1* and *DRB3, 4,* or *5*). The nomenclature of the HLA locus takes into account the enormous polymorphism identified by serologic and molecular methods. Thus, based on modern molecular typing, individual alleles may be called *HLA-A∗0201,* referring to the 01 subtype of *HLA-A2,* or *HLA-DRB1∗0401,* referring to the 01 subtype of the *DR4B1* gene, and so on.

The mouse MHC, located on chromosome 17, occupies about 2000 kb of DNA, and the genes are organized in an order slightly different from those in the human MHC. One of the mouse class I genes *(H-2K)* is centromeric to the class II region, but the other class I genes are telomeric to the class II region. There are three mouse class I MHC genes called *H-2K, H-2D,* and *H-2L,* encoding three different class I MHC proteins, K, D, and L. These genes are homologous to the human *HLA-A, -B,* and *-C* genes. The MHC alleles of particular inbred strains of mice are designated by lowercase letters (e.g., *a*, *b*), named for the whole set of MHC genes of the mouse strain in which they were first identified. In the parlance of mouse geneticists, the allele of the *H-2K* gene in a strain with the k-type MHC is called K^k (pronounced *K of k*), whereas the allele of the *H-2K* gene in a strain with d-type MHC is called K^d *(K of d)*. Similar terminology is used for *H-2D* and *H-2L* alleles. Mice have two class II MHC loci called *I-A* and *I-E,* which encode the I-A and I-E molecules, respectively. These loci are the Ir genes discussed earlier. The mouse class II genes are homologous to human *HLA-DP, DQ,* and *DR* genes. The *I-A* allele found in the inbred mouse strain with the K^{k} and D^{k} alleles is called $I\text{-}A^{k}$ (pronounced *I-A of k*). Similar terminology is used for the *I-E* allele. As in humans, there are actually two different genes, designated *A* and *B,* in the *I-A* and *I-E* loci that encode the α and β chains of each class II MHC molecule.

The set of MHC alleles present on each chromosome is called an MHC **haplotype.** For instance, an HLA haplotype of an individual could be HLA-A24, B35, C3, DRB12, DPB1 DQB3, and so on (using the simpler nomenclature for HLA alleles). All heterozygous individuals, of course, have two HLA haplotypes. Inbred mice, being homozygous, have a single haplotype. Thus, the haplotype of an $H\text{-}2^{d}$ mouse is $H\text{-}2K^{d}$ $I\text{-}A^{d}$ $I\text{-}E^{d}$ D^{d} L^{d}. The MHC genes are tightly linked, so that haplotypes are inherited en bloc, and individuals will usually express all of the MHC alleles in the two haplotypes inherited from their parents.



Proteasome
DP
DM
TAP2
DQ
DR
B1 A1
A B
TAP1
A2 B1 A1
B1 B3 A
Tapasin
DOA
DOB
Class II Region
0
200
400
600
800
1000
Factor B
TNF
C4B
C4A
C2
LTβ
LTα
MIC-B
Class III Region
1000
1200
1400
1600
1800
2000
MIC-A
HLA-B
HLA-C
HLA-E
2000
2200
2400
2600
2800
3000
Class I Region
HLA-A
HLA-G
HLA-F
3000
3200
3400
3600
3800
4000
kb

FIGURE 6.7 Map of the human major histocompatibility complex. The genes located within the human MHC locus are illustrated. In addition to the class I and class II *MHC* genes, *HLA-E, HLA-F,* and *HLA-G* and the *MIC* genes encode class I–like molecules, many of which are recognized by NK cells. C4, C2, and factor B are complement proteins; tapasin, DM, DO, TAP, and proteasome subunits are proteins involved in antigen processing, discussed later in the chapter; LTα, LTβ, and TNF are cytokines. Many pseudogenes and genes whose roles in immune responses are not established are located in the HLA complex but are not shown to simplify the map. *HLA,* Human leukocyte antigen; *LT,* lymphotoxin; *TAP,* transporter associated with antigen processing; *TNF,* tumor necrosis factor.

Expression of MHC Molecules

Because MHC molecules are required to present antigens to T lymphocytes, the expression of these proteins in a cell determines whether foreign (e.g., microbial) antigens in that cell will be recognized by T cells. There are several important features of the expression of MHC molecules that contribute to their role in protecting individuals from different microbial infections.

Class I MHC molecules are expressed on virtually all nucleated cells, whereas class II MHC molecules are expressed only on DCs, B lymphocytes, macrophages, thymic epithelial cells, and a few other cell types. This pattern of MHC expression is linked to the functions of class I–restricted CD8$^+$ and class II–restricted CD4$^+$ T cells. As discussed earlier, CD8$^+$ CTLs kill cells infected with intracellular microbes, such as viruses, as well as tumors that express tumor antigens, and any nucleated cell can harbor a virus or develop into cancer. Thus, the expression of class I MHC molecules on nucleated cells provides a display system for viral and tumor antigens, so these antigens can be recognized by CTLs and the antigen-producing cells can be killed. In contrast, class II–restricted CD4$^+$ helper T lymphocytes have a set of functions that require recognizing antigen presented by a more limited number of cell types, and class II MHC molecules are expressed mainly on these cell types. Differentiated CD4$^+$ helper T lymphocytes function mainly to activate (or help) macrophages to eliminate extracellular microbes that have been phagocytosed and to help B lymphocytes make antibodies that also eliminate extracellular microbes. In order to initiate an immune response, naive CD4$^+$ and CD8$^+$ T cells need to recognize antigens that are captured and presented by DCs in lymphoid organs, which express both class I and class II MHC molecules. Thymic epithelial cells also express both class I and class II molecules, and antigen display by these cells is important in the process of selection of maturing T lymphocytes (see Chapter 8).

The expression of MHC molecules is increased by cytokines produced during both innate and adaptive immune responses.

Although class I MHC molecules are constitutively expressed on nucleated cells, their expression is increased by the type I IFNs IFN-α and IFN-β, which are produced during the early innate immune response to many viruses (see Chapter 4). Thus, innate immune responses to viruses increase the expression of the MHC molecules that display viral antigens to virus-specific T cells. This is one of the mechanisms by which innate immunity stimulates adaptive immune responses. The expression of class I molecules is also increased by IFN-γ.

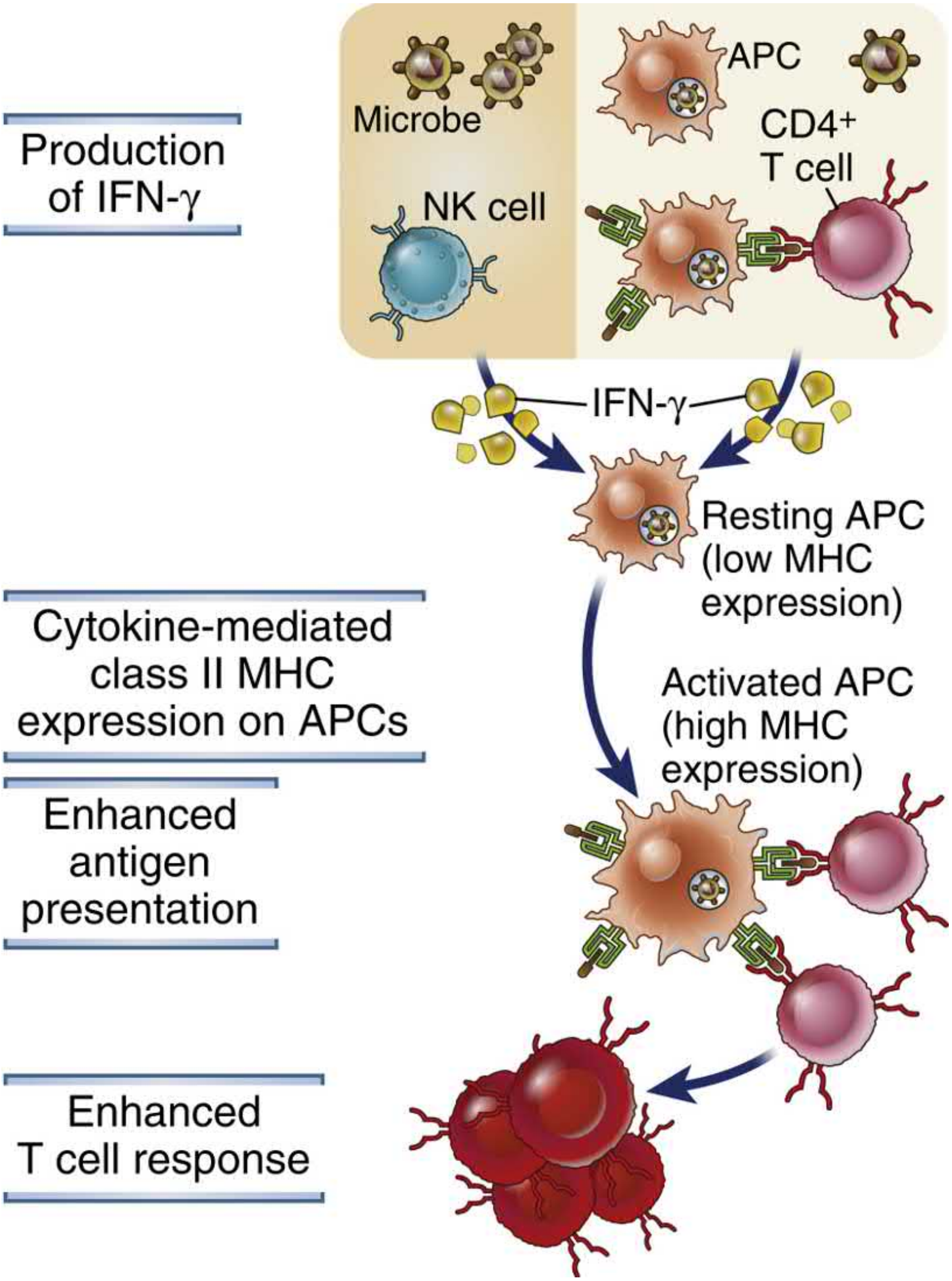

Production of IFN-γ
Microbe
NK cell
APC
CD4+ T cell
IFN-γ
Resting APC (low MHC expression)
Cytokine-mediated class II MHC expression on APCs
Activated APC (high MHC expression)
Enhanced antigen presentation
Enhanced T cell response

FIGURE 6.8 Enhancement of class II major histocompatibility complex molecule expression by interferon-γ. IFN-γ, produced by NK cells and other cell types during innate immune reactions to microbes or by T cells during adaptive immune reactions, stimulates class II MHC expression on

APCs and thus enhances the activation of CD4$^+$ T cells. IFN-γ and type I IFNs have a similar effect on the expression of class I MHC molecules and the activation of CD8$^+$ T cells. *APC,* Antigen-presenting cell; *IFN,* interferon; *NK,* natural killer.

The expression of class II MHC molecules is regulated by cytokines and other signals in different cells. IFN-γ is the principal cytokine involved in stimulating expression of class II molecules in APCs such as DCs and macrophages (Fig. 6.8). IFN-γ may be produced by natural killer (NK) cells during early innate immune reactions and by antigen-activated T cells during later adaptive immune reactions. Thus, the ability of IFN-γ to increase class II MHC expression provides a mechanism by which innate immunity promotes adaptive immunity and also an amplification mechanism in adaptive immunity. B lymphocytes constitutively express class II molecules and can increase expression in response to antigen recognition and cytokines produced by helper T cells, thus enhancing antigen presentation to helper cells (see Chapter 12). IFN-γ can also increase the expression of MHC molecules on vascular endothelial cells and other nonimmune cell types; the role of these cells in antigen presentation to T lymphocytes is unclear, as mentioned earlier. Some cells, such as neurons, never appear to express class II MHC molecules. After activation, human but not mouse T cells express class II MHC molecules; however, no cytokine has been identified in this response and its functional significance is unknown.

The amount of transcription is the major determinant of the level of MHC molecule synthesis and expression on the cell surface. Cytokines enhance MHC expression by stimulating the transcription of class I and class II genes in a wide variety of cell types. These effects are mediated by the binding of cytokine-activated transcription factors to DNA sequences in the promoter regions of MHC genes. Several transcription factors may be assembled and bind a protein called the class II transcription activator (CIITA), and the entire complex binds to the class II promoter and promotes efficient transcription of the gene. By keeping the complex of transcription factors together, CIITA functions as a master regulator of class II gene expression. Mutations in CIITA or the associated transcription factors have been identified as the cause of human immunodeficiency diseases associated with defective expression of MHC molecules. The best studied of these disorders is **bare lymphocyte syndrome** (see Chapter 21). Knockout mice lacking CIITA also show reduced or absent class II expression on DCs and B lymphocytes and an inability of IFN-γ to induce class II on all cell types.

The expression of many of the proteins involved in antigen processing and presentation is coordinately regulated. For instance, IFN-γ increases the transcription not only of class I and class II genes but also of several genes whose products are required for class I MHC assembly and peptide display, such as genes encoding the transporter associated with antigen processing (TAP) and some of the subunits of proteasomes, discussed later in this chapter.

In addition to transcriptional regulation, the level of class II MHC expression is controlled by the level of ubiquitination-dependent degradation, discussed later in the context of antigen processing.

Structure of MHC Molecules

Biochemical studies of MHC molecules culminated in the solution of the crystal structures for the extracellular portions of human class I and class II MHC molecules. Subsequently, many MHC molecules with bound peptides have been crystallized and analyzed in detail. Because of these advances, we now understand how MHC molecules bind and display peptides. In this section, we first summarize the functionally important biochemical features that are common to class I and class II MHC molecules. We then describe the structures of class I and class II proteins, pointing out their main similarities and differences (Table 6.3).

General Properties of MHC Molecules

All MHC molecules share certain structural characteristics that are critical for their role in peptide display and antigen recognition by T lymphocytes.

- ***Each MHC molecule consists of an extracellular peptide-binding cleft, followed by an immunoglobulin (Ig)-like domain and transmembrane and cytoplasmic domains.*** Class I MHC molecules are composed of one polypeptide chain encoded in the MHC and a second, non-MHC–encoded chain, whereas class II MHC molecules are made up of two MHC-encoded polypeptide chains. Despite this difference, the overall three-dimensional structures of class I and class II molecules are similar.

TABLE 6.3

Feature	**Class I MHC**	**Class II MHC**
Polypeptide chains	α and β_2-microglobulin	α and β
Locations of polymorphic residues	α1 and α2 domains	β1 > α1 domains
Binding site for T cell coreceptor	CD8 binds mainly to the α3 domain	CD4 binds to a pocket created by parts of α2 and β2 domains
Size of peptide-binding cleft	Accommodates peptides of 8–11 residues	Accommodates peptides of 10–30 residues or more
Nomenclature		
Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP
Mouse	H-2K, H-2D, H-2L	I-A, I-E

HLA, Human leukocyte antigens.

- ***The polymorphic amino acid residues of MHC molecules are located in and adjacent to the peptide-binding cleft.*** This cleft (also called a groove) is formed by the folding of the amino termini of the MHC-encoded proteins and is composed of paired *α* helices forming the two walls of the cleft, resting on a floor made up of an eight-stranded β-pleated sheet. The polymorphic residues, which are the amino acids that vary among different MHC alleles, are located in the floor and walls of this cleft. This portion of the MHC molecule binds peptides for display to T cells, and the antigen receptors of T cells interact with the displayed peptide and also with the *α* helices of the MHC molecules (see Fig. 6.1). Because of amino acid variability in this region, different MHC molecules bind and display different peptides and are recognized by the antigen receptors of different T cells.

- ***The nonpolymorphic Ig-like domains of class II and class I MHC molecules contain binding sites for the T cell molecules CD4 and CD8, respectively.*** CD4 and CD8 are expressed on distinct subpopulations of mature T lymphocytes and participate, together with antigen receptors, in responses to peptide-MHC complexes. For this reason, CD4 and CD8 are called T cell coreceptors (see Chapter 7). CD4 binds selectively to class II MHC molecules, and CD8 binds to class I MHC molecules. $CD4^+$ **helper T cells recognize class II MHC molecules displaying peptides, whereas $CD8^+$** T cells recognize class I MHC molecules with bound peptides. Stated differently, $CD4^+$ T cells are class II MHC restricted and $CD8^+$ T cells are class I MHC restricted.

Class I MHC Molecules

Class I MHC molecules consist of two noncovalently linked polypeptide chains, an MHC-encoded 44- to 47-kD α chain (or heavy chain) and a non–MHC-encoded 12-kD subunit called β2-microglobulin (Fig. 6.9). About three-quarters of the α chain polypeptide is extracellular; a short hydrophobic segment spans the plasma membrane, and the carboxy-terminal residues are located in the cytoplasm. The amino-terminal $\alpha 1$ and $\alpha 2$ segments of the α chain, each approximately 90 residues long, interact to form a platform of an eight-stranded, antiparallel β-pleated sheet supporting two parallel strands of α helix. This forms the peptide-binding cleft of class I MHC molecules. Its size is large enough (~25 Å × 10 Å × 11 Å) to bind peptides of 8 to 11 amino acids in a flexible, extended conformation. The ends of the class I peptide–binding cleft are closed so that larger peptides cannot be accommodated. Therefore, native globular proteins have to be converted into fragments that are small enough and in an extended linear shape so they can bind to MHC molecules and be recognized by T cells (described later). The polymorphic residues of class I MHC molecules are confined to the $\alpha1$ and $\alpha2$ domains, where they contribute to variations among different class I alleles in peptide binding and T cell recognition (Fig. 6.10). The $\alpha3$ segment of the α chain folds into an Ig domain whose amino acid sequence is conserved among all class I MHC molecules. This segment contains most of the binding site for CD8, but β2-microglobulin and a small part of the nonpolymorphic C-terminal portion of the $\alpha2$ domain also contribute. At the carboxy-terminal end of the $\alpha3$ segment is a stretch of approximately 25 hydrophobic amino acids that traverses the lipid bilayer of the plasma membrane. Immediately after this are approximately 30 residues located in the cytoplasm, which include a cluster of basic amino acids that interact with phospholipid head groups of the inner leaflet of the lipid bilayer and anchor the MHC molecule in the plasma membrane.

β2-Microglobulin, the light chain of class I MHC molecules, is encoded by a gene outside the MHC and is named for its electrophoretic mobility (β2), small size (micro), and solubility (globulin). It interacts noncovalently with the $\alpha3$ domain of the α chain. Like the $\alpha3$ segment, β2-microglobulin is structurally homologous to an Ig domain and is invariant among all class I MHC molecules.

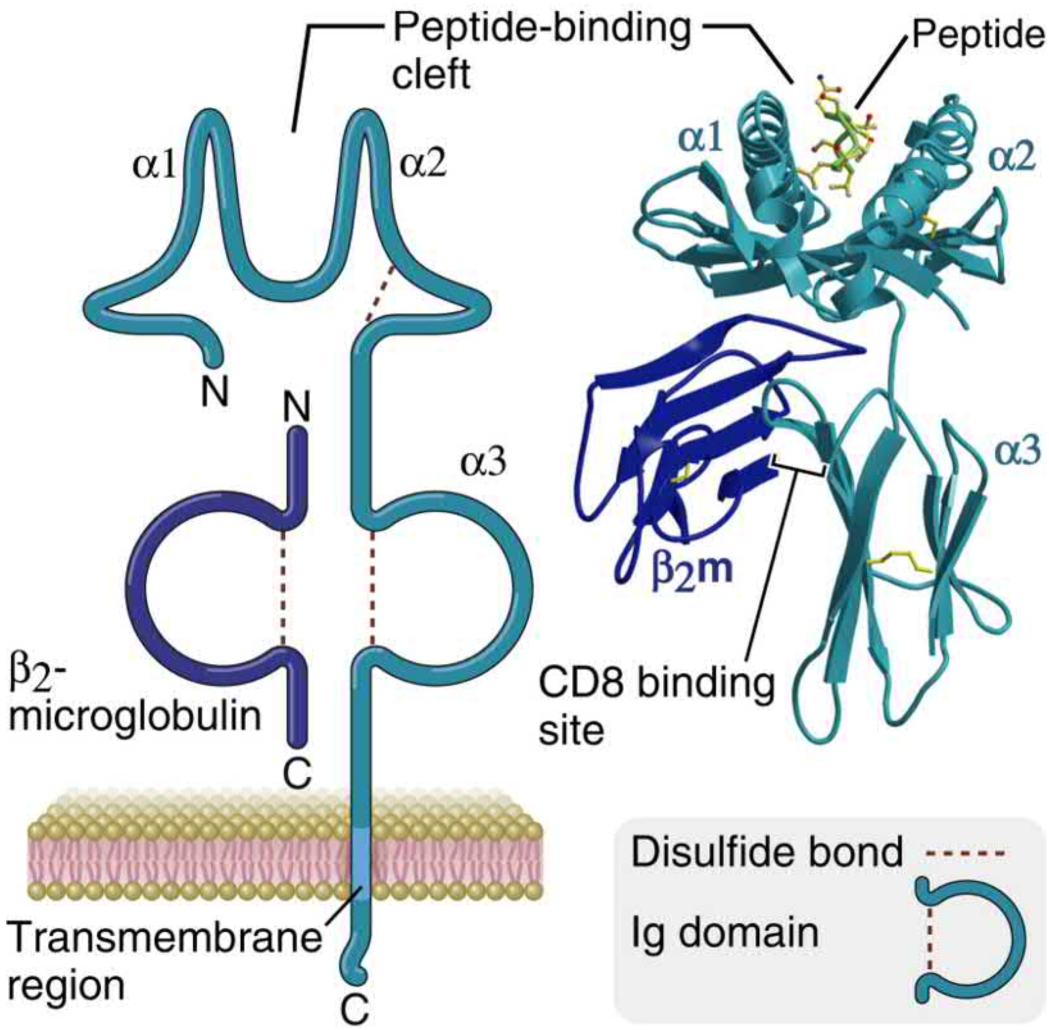

Peptide-binding cleft
Peptide
α1
α2
α1
α2
N
N
α3
α3
β2m
β2-microglobulin
CD8 binding site
C
Transmembrane region
C
Disulfide bond
Ig domain

FIGURE 6.9 Structure of a class I major histocompatibility complex molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class I MHC molecules are composed of a polymorphic α chain noncovalently attached to the nonpolymorphic β2-microglobulin (β2m). The α chain is glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-B27 molecule with a bound peptide, resolved by x-ray crystallography. *HLA,* Human leukocyte antigen; *Ig,* immunoglobulin.

Courtesy Dr. P. Bjorkman, California Institute of Technology, Pasadena, CA.

The fully assembled class I MHC molecule is a trimeric complex consisting of an α chain, β 2-microglobulin, and bound peptide, and stable expression of class I MHC molecules on cell surfaces requires the presence of all three components of the complex. The reason for this is that the interaction of the α chain with β2-microglobulin is stabilized by binding of peptide antigens to the cleft formed by the α1 and α2 segments, and, conversely, the binding of peptide is strengthened by the interaction of β2-microglobulin with the α chain. Because peptides are needed to stabilize the MHC molecules and unstable complexes are degraded, only potentially useful peptide-loaded MHC molecules are expressed on cell surfaces.

Most individuals are heterozygous for MHC genes and therefore express six different class I MHC molecules on every cell, containing α chains encoded by the two inherited alleles of *HLA-A, B,* and *C* genes.

Class II MHC Molecules

Class II MHC molecules are composed of two noncovalently associated polypeptide chains, a 32- to 34-kD α chain, and a 29- to 32-kD β chain (Fig. 6.11). Unlike class I MHC molecules, the genes encoding both chains of class II MHC molecules are polymorphic and located in the MHC locus.

The amino-terminal α1 and β1 segments of the class II chains interact to form the peptide-binding cleft, which is structurally similar to the cleft of class I MHC molecules. Four strands of the floor of the cleft and one of the α-helical walls are formed by the α1 segment, and the other four strands of the floor and the second wall are formed by the β1 segment. The polymorphic residues are located in the α1 and β1 segments, in and around the peptide-binding cleft, as in class I MHC molecules (see Fig. 6.10). In human class II MHC molecules, most of the polymorphism is in the β chain. The ends of the peptide-binding cleft of class II MHC molecules are open, so peptides of 10 to over 30 residues can bind.

The α2 and β2 segments of class II MHC molecules, like class I α3 and β2-microglobulin, are folded into Ig domains and are nonpolymorphic—that is, they do not vary among alleles of a particular class II gene. Both the α2 and β2 domains of class II MHC molecules contribute to a concavity that accommodates a protrusion of the CD4 protein, thus allowing binding to occur. The carboxy-terminal ends of the α2 and β2 segments continue into short connecting regions followed by approximately 25 amino acid stretches of hydrophobic transmembrane residues. In both chains, the transmembrane regions end with clusters of basic amino acid residues, followed by short hydrophilic cytoplasmic tails.

The fully assembled class II MHC molecule is a trimer consisting of one α chain, one β chain, and a bound antigenic peptide, and stable expression of class II MHC molecules on cell surfaces requires the presence of all three components of the complex. As in class I MHC molecules, this ensures that the MHC molecules that end up on the cell surface are the molecules that are carrying out their normal function of peptide display.

Humans inherit, from each parent, one *DPA* and one *DPB* gene encoding, respectively, the α and β chains of an HLA-DP molecule; one functional *DQA* and one *DQB* gene; one *DRA* and one or two functional *DRB* genes. Thus, each heterozygous individual expresses six to eight pairs of class II MHC α and β chain molecules, one set each of *DP* and *DQ,* and one or two of *DR*. Typically, there is not much pairing of MHC proteins from different loci (i.e., DRα with DQβ, and so on), and each haplotype tends to be inherited as a single unit. However, because some haplotypes contain extra *DRB* loci that produce β chains that assemble with DRα, and some DQα molecules encoded on one chromosome can associate with DQβ molecules produced from the other chromosome, the total number of expressed class II MHC molecules on the cells of some individuals may be more than eight.

Binding of Peptides to MHC Molecules

After the demonstration that the immunogenicity of proteins depends on the ability of their peptides to be displayed by MHC molecules, considerable effort has been devoted to elucidating the molecular basis of peptide-MHC interactions and the characteristics of peptides that allow them to bind to MHC molecules. These studies initially relied on functional assays of helper T cells and CTLs responding to APCs that were incubated with different peptides. Direct binding of MHC molecules and peptides has been studied with purified MHC molecules and radioactively or fluorescently labeled peptides in solution, using methods such as equilibrium dialysis and gel filtration. X-ray crystallographic analysis of peptide-MHC complexes has provided definitive information about how peptides sit in the clefts of MHC molecules and about the residues of each that participate in this binding. This information has been used to generate computer algorithms that can predict peptides of any given protein that are most likely to bind to MHC molecules. This information can theoretically be used to develop vaccines specific for microbial proteins or mutated tumor proteins (see Chapter 18). In the section that follows, we summarize the key features of the interactions between peptides and class I or class II MHC molecules.

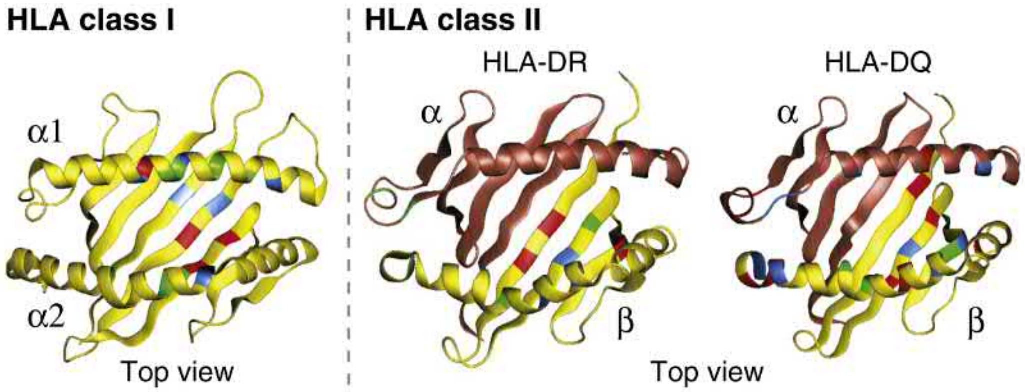


FIGURE 6.10 Polymorphic residues of major histocompatibility complex molecules. The polymorphic residues of class I and class II MHC molecules are located in the peptide-binding clefts and the α helices around the clefts. The regions of greatest variability among different human leukocyte antigens *(HLA)* alleles are indicated in *red,* of intermediate variability in *green,* and of the lowest variability in *blue*.

Reproduced with permission from Margulies DH, Natarajan K, Rossjohn J, McCluskey J. Major histocompatibility complex [MHC] molecules: structure, function, and genetics. In Paul WE, ed. *Fundamental Immunology.* 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2008.

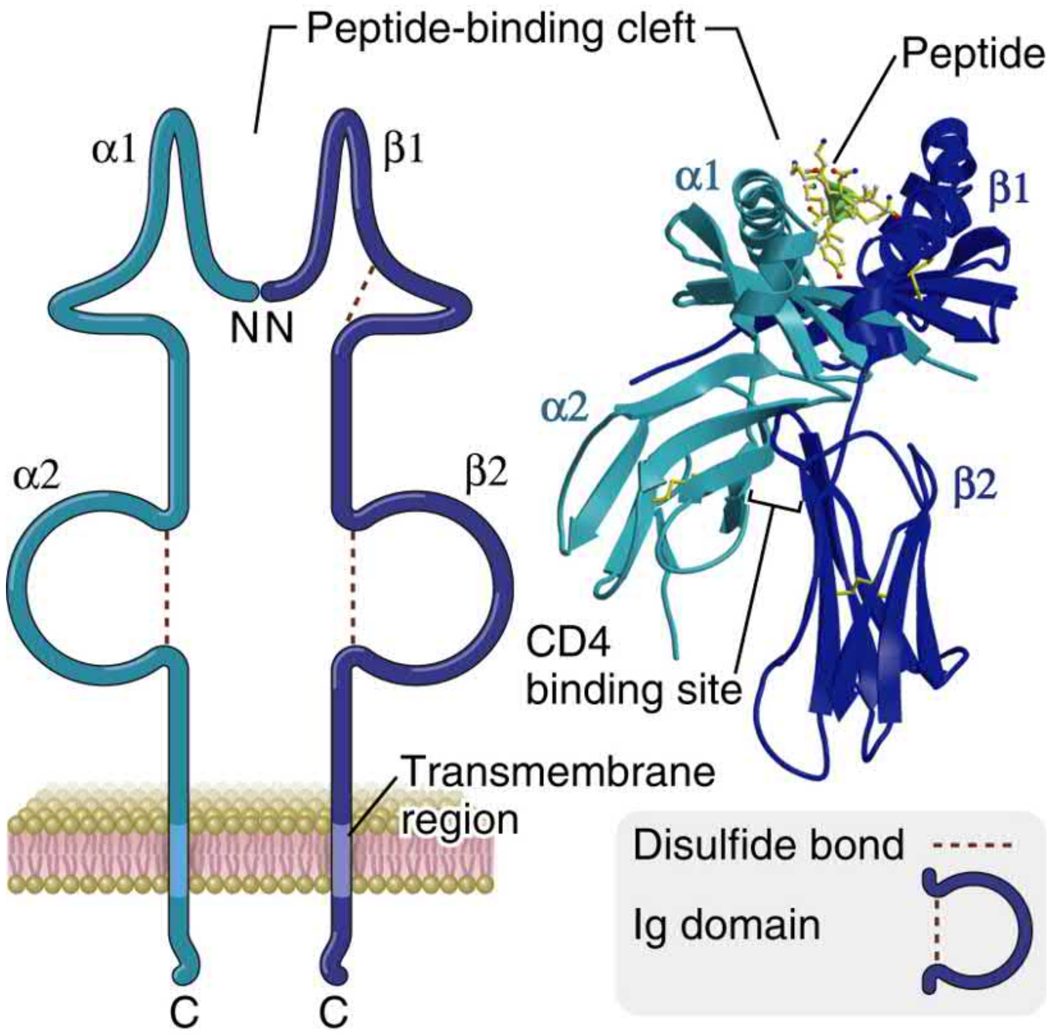

Peptide-binding cleft
Peptide
α1
β1
NN
α2
β2
α1
β1
α2
β2
CD4 binding site
Transmembrane region
C
C
Disulfide bond
Ig domain

FIGURE 6.11 Structure of a class II major histocompatibility complex molecule. The schematic diagram *(left)* illustrates the different regions of the MHC molecule (not drawn to scale). Class II MHC molecules are composed of a polymorphic α chain noncovalently attached to a polymorphic β chain. Both chains are glycosylated; carbohydrate residues are not shown. The ribbon diagram *(right)* shows the structure of the extracellular portion of the HLA-DR1 molecule with a bound peptide, resolved by x-ray crystallography. *HLA,* Human leukocyte antigen; *Ig,* immunoglobulin.

Courtesy Dr. P. Bjorkman, California Institute of Technology, Pasadena, CA.

Characteristics of Peptide-MHC Molecule Interactions

MHC molecules show a broad specificity for peptide binding, in contrast to the fine specificity of antigen recognition by the antigen receptors of lymphocytes. In other words, a single MHC allele (e.g., HLA-A2) can present any one of many different peptides to T cells, but a single T cell will recognize only one of these many possible HLA-A2–peptide complexes. There are several important features of the interactions of MHC molecules and antigenic peptides.

- ***Each class I and class II MHC molecule has a single peptide-binding cleft that binds one peptide at a time, but each MHC molecule can bind many different peptides.*** One of the earliest lines of evidence supporting this conclusion was the experimental result that different peptides that bind to the same MHC molecule can competitively inhibit one another's presentation, implying that there is only a single peptide-binding cleft in every MHC molecule. The solution of the crystal structures of class I and class II MHC molecules confirmed the presence of a single peptide-binding cleft in these molecules (see Figs. 6.9 and 6.11). It is not surprising that a single MHC molecule can bind multiple peptides, because each individual contains only a few different MHC molecules (six class I and eight or a few more class II molecules in a heterozygous individual), and these must be able to present peptides from the enormous number of protein antigens that one is likely to encounter.
- ***The peptides that bind to MHC molecules share structural features that promote this interaction.*** One of these features is the size of the peptide—class I MHC molecules can accommodate peptides that are 8 to 11 residues long, and class II MHC molecules bind peptides that may be 10 to 30 residues long or even longer, the optimal length for fitting

into the class II MHC cleft being 12 to 16 residues. In addition, peptides that bind to a particular MHC molecule contain amino acid residues that allow complementary interactions between the peptide and that MHC molecule. Some of the amino acid residues that promote binding to MHC molecules are described later, when we discuss the structural basis of peptide-MHC interactions. The residues of a peptide that bind to MHC molecules are distinct from those that are recognized by T cells.

- ***MHC molecules acquire their peptide cargo during their biosynthesis and assembly inside cells.*** Therefore, MHC molecules display peptides derived from microbial antigens that are inside host cells, and this is why MHC-restricted T cells are able to recognize microbes that infect or are ingested into cells. The mechanisms and significance of these processes are discussed later in this chapter.
- ***The association of peptides and MHC molecules is a saturable interaction with a very slow off-rate.*** In a cell, several chaperones and enzymes facilitate the binding of peptides to MHC molecules (described later). Once formed, most peptide-MHC complexes are stable, and kinetic dissociation constants are indicative of long half-lives that range from hours to many days. This extraordinarily slow off-rate of peptide dissociation from MHC molecules ensures that after an MHC molecule has acquired a peptide, it will display the peptide long enough to maximize the chance that a particular T cell will find the peptide it can recognize and initiate a response.
- ***Very small numbers of peptide-MHC complexes are capable of activating specific T lymphocytes.*** Because APCs continuously present peptides derived from all the proteins they encounter, only a very small fraction of cell surface peptide–MHC complexes will contain the same peptide. It has been estimated that as few as 100 complexes of a particular peptide with a class II MHC molecule on the surface of an APC can initiate a specific T cell response.

This represents less than 0.1% of the total number of class II molecules likely to be present on the surface of the APC.

- ***The MHC molecules of an individual can bind and display foreign peptides (e.g., those derived from microbial proteins) and peptides derived from the proteins of that individual (self antigens).*** In fact, most of the peptides being displayed normally by APCs are derived from self proteins. The inability of MHC molecules to discriminate between self and foreign peptides raises the question of why we normally do not develop immune responses against self proteins. The answer is that self peptide–MHC complexes do not induce autoimmunity because T cells specific for such complexes are killed or inactivated. In fact, T cells with receptors for self antigens must recognize self peptides displayed by self MHC molecules in order to be eliminated or made unresponsive. These processes ensure that T cells are normally tolerant to self antigens (see Chapter 15).

Structural Basis of Peptide Binding to MHC Molecules

The binding of peptides to MHC molecules is a noncovalent interaction mediated by residues both in the peptides and in the clefts of the MHC molecules. As we will discuss later, protein antigens are proteolytically cleaved in APCs to generate the peptides that will be bound and displayed by MHC molecules. These peptides bind to the clefts of MHC molecules in an extended conformation. Once bound, the peptides and their associated water molecules fill the clefts, making extensive contacts with the amino acid residues that form the β strands of the floor and the α helices of the walls of the cleft (Fig. 6.12).

In most MHC molecules, the β strands in the floor of the cleft contain pockets where side chains of amino acid residues of peptides bind. Many class I MHC molecules have a hydrophobic pocket that recognizes one of the following hydrophobic amino acids—valine, isoleucine, leucine, or methionine—at the C-terminal end of the peptide. Some class I molecules have a predilection for peptides with a basic residue (lysine or arginine) at the C terminus. In addition, other amino acid residues of a peptide may contain side chains that fit into specific pockets and bind to complementary amino acids in the MHC molecule through electrostatic interactions (salt bridges), hydrogen bonding, or van der Waals interactions. The residues of the peptide that fit into the MHC pockets are called anchor residues because they contribute most to the binding—or anchoring—of the peptide in the cleft of the MHC molecule. Each MHC-binding peptide usually contains only one or two anchor residues, and this presumably allows greater variability in the other residues of the peptide, which are the residues that are recognized by specific T cells. In the case of some peptides binding to MHC molecules, especially class II MHC molecules, specific interactions of peptides with the α-helical sides of the MHC cleft also contribute to peptide binding by forming hydrogen bonds or charge interactions. Class II MHC molecules accommodate larger peptides than class I MHC molecules. These longer peptides extend at either end beyond the floor of the cleft.

Because many of the residues in and around the peptide-binding cleft of MHC molecules are polymorphic (i.e., they differ among various MHC alleles), different alleles favor the binding of different peptides. This is the structural basis for the function of MHC genes as immune response genes; only individuals whose MHC molecules can bind a particular peptide and display it to T cells can respond to that peptide.

The antigen receptors of T cells recognize both the antigenic peptides and the MHC molecules, with the peptide being responsible for the fine specificity of antigen recognition and the MHC residues accounting for the MHC restriction of the T cells. A portion of the bound peptide is exposed from the open top of the cleft of the MHC molecule, and the amino acid side chains of this portion of the peptide are recognized by the antigen receptors of specific T cells. The same T cell receptor also interacts with polymorphic residues of the α helices of the MHC molecule itself (see Fig. 6.1). Predictably, variations in either the peptide antigen or the peptide-binding cleft of the MHC molecule will alter presentation of that peptide or its recognition by T cells.

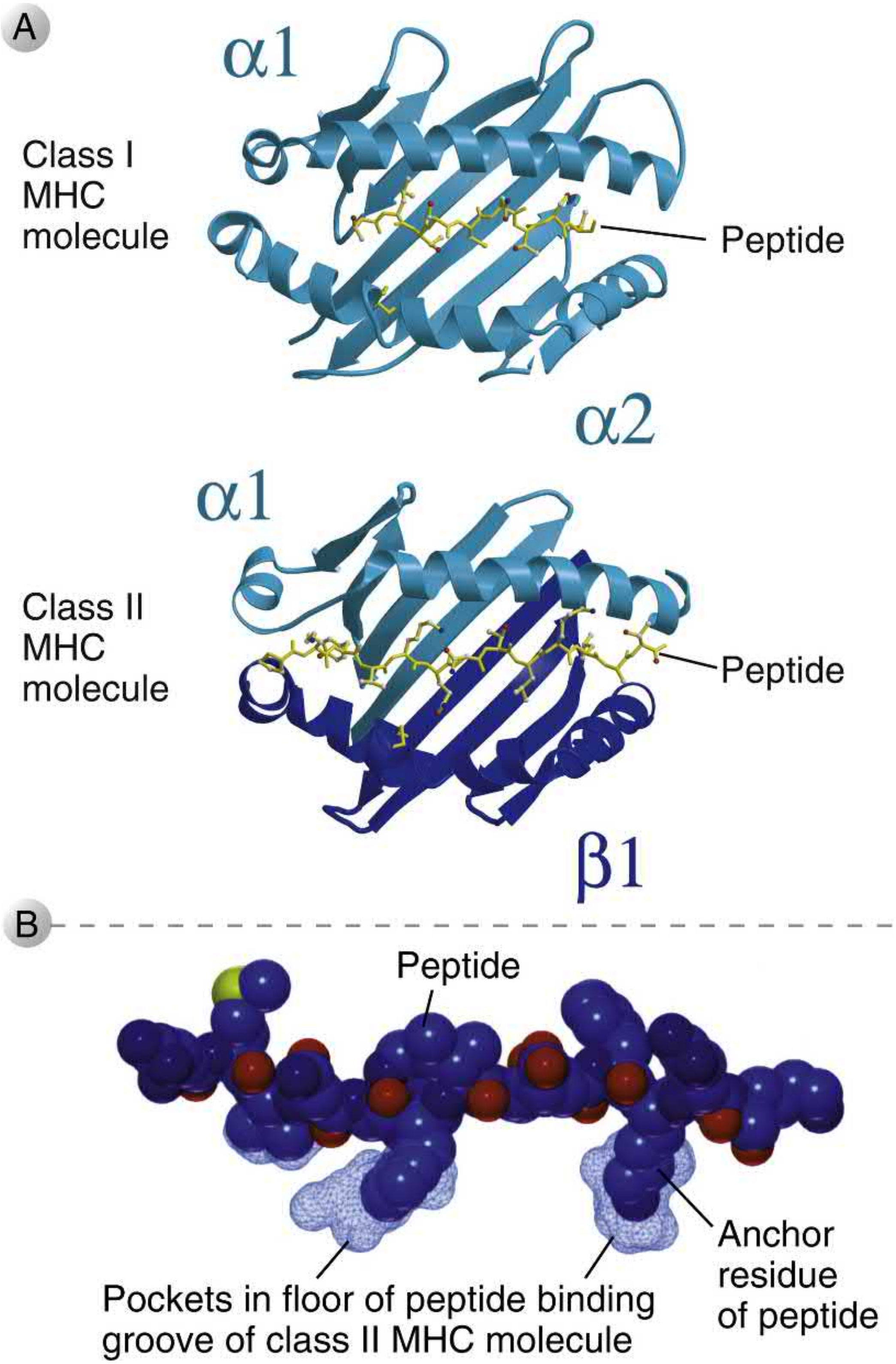

A
α1
Class I MHC molecule
Peptide
α2
α1
Class II MHC molecule
Peptide
β1
B
Peptide
Pockets in floor of peptide binding groove of class II MHC molecule
Anchor residue of peptide

FIGURE 6.12 Peptide binding to major histocompatibility complex molecules. **A,** These

top views of the crystal structures of MHC molecules show how peptides lie in the peptide-binding clefts. The class I MHC molecule shown is HLA-A2, and the class II MHC molecule is HLA-DR1. The cleft of the class I MHC molecule is closed, whereas that of the class II MHC molecule is open. As a result, class II MHC molecules accommodate longer peptides than class I MHC molecules. **B,** The side view of a cutout of a peptide bound to a class II MHC molecule shows how anchor residues of the peptide hold it in the pockets in the cleft of the MHC molecule. *HLA,* Human leukocyte antigen.

A, Courtesy Dr. P. Bjorkman, California Institute of Technology, Pasadena. *B,* From Scott CA, Peterson PA, Teyton L, Wilson IA: Crystal structures of two I-Ad–peptide complexes reveal that high affinity can be achieved without large anchor residues. *Immunity.* 1998;8:319–329. Copyright © 1998, with permission from Elsevier Science.

Because MHC molecules can bind only linear peptides but microbial and other protein antigens are large molecules in various folded conformations, there must a mechanism by which these proteins are converted into peptides that can bind to MHC molecules. The mechanism is called **antigen processing** and is the focus of the remainder of the chapter.

Processing of Protein Antigens

The pathways of antigen processing convert protein antigens present in the cytosol or internalized from the extracellular environment into peptides and load these peptides onto MHC molecules for display to T lymphocytes (Fig. 6.13) **.** The mechanisms of antigen processing have evolved to generate peptides that have the structural characteristics required for associating with MHC molecules, and to place these peptides in the same cellular location as newly synthesized MHC proteins with available peptide-binding clefts. Peptide binding to MHC molecules occurs before cell surface expression and is an integral component of the biosynthesis and assembly of MHC molecules. In fact, as mentioned earlier, peptide association is required for the assembly and surface expression of stable class I and class II MHC molecules.

Proteins that are present in the cytosol are degraded by proteasomes to yield peptides that are displayed on class I MHC molecules, whereas proteins that are ingested from the extracellular environment and sequestered in vesicles are degraded in lysosomes (or late endosomes) to generate peptides that are presented on class II MHC molecules (see Fig. 6.13 and Table 6.4) **.** In the process of cross-presentation, described later, antigens are ingested into vesicles and then transported into the cytosol, where they are processed for display by class I MHC molecules. Thus, the site of proteolysis is the key determinant of the MHC molecules, class I or class II, to which the generated peptides will bind. As we have discussed previously, the function of CD8$^+$ CTLs is to kill cells producing foreign antigens in the cytosol and the function of CD4$^+$ T cells is to activate macrophages and B cells, which may have ingested microbes and protein antigens. The pathways of antigen processing play a key role in determining the types of microbes and protein antigens that these classes of T cells recognize and to which they respond. We first describe these two pathways of antigen processing and then their functional significance.

The Class I MHC Pathway for Processing and Presentation of Cytosolic Proteins

The sequence of events in antigen presentation on class I MHC molecules is illustrated in Fig. 6.14, and the individual steps are described next.

Sources of Protein Antigens Degraded in Proteasomes

Microbial proteins present in the cytosol that undergo proteasomal degradation are derived from microbes that either produce antigens in the cytosol of cells or whose antigens are transferred to the cytosol. The same principles apply to tumor antigens. These cytosolic antigens come from a number of sources.

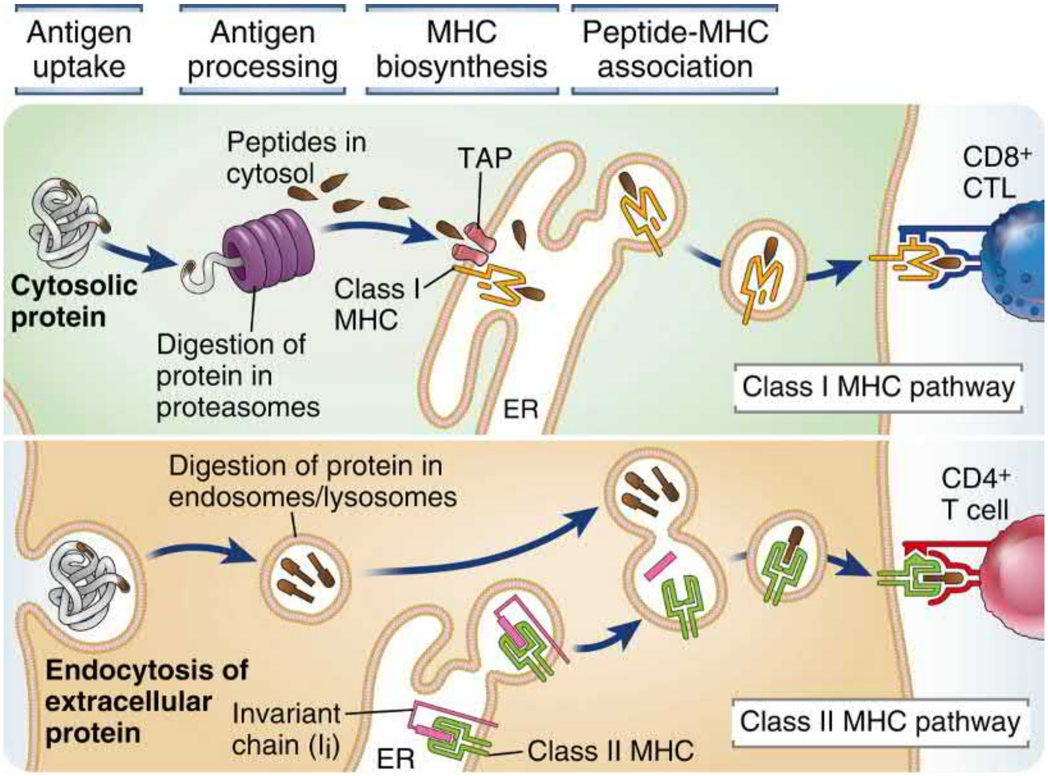
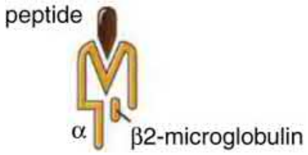
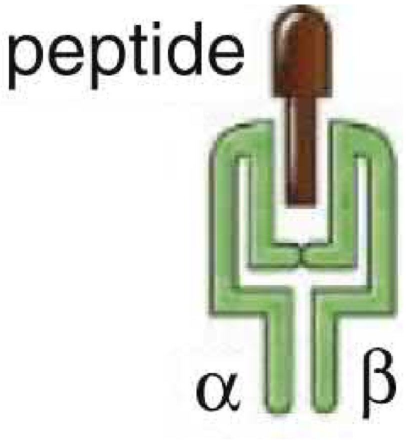

Antigen uptake
Antigen processing
MHC biosynthesis
Peptide-MHC association
Peptides in cytosol
TAP
CD8+ CTL
Cytosolic protein
Class I MHC
Digestion of protein in proteasomes
ER
Class I MHC pathway
Digestion of protein in endosomes/lysosomes
CD4+ T cell
Endocytosis of extracellular protein
Invariant chain (Ii)
ER
Class II MHC
Class II MHC pathway

FIGURE 6.13 Pathways of antigen processing and presentation.In the class I MHC pathway *(top panel),* protein antigens in the cytosol are processed by proteasomes, and peptides are transported into the ER, where they bind to class I MHC molecules. In the class II MHC pathway *(bottom panel),* protein antigens that are degraded in lysosomes bind to class II MHC molecules. Details of these processing pathways are shown in Figs. 6.14 and 6.15. *CTL,* Cytotoxic T lymphocytes; *ER,* endoplasmic reticulum; *TAP,* transporter associated with antigen processing.

TABLE 6.4

Comparative Features of Class I and Class II Major Histocompatibility Complex Pathways of Antigen Processing and Presentation

Feature	Class I MHC Pathway	Class II MHC Pathway
Composition of stable peptide-MHC complex	 Polymorphic α chain, β2-microglobulin, peptide	 Polymorphic α and β chains, peptide
Types of APCs	All nucleated cells	Dendritic cells, mononuclear phagocytes, B lymphocytes, endothelial cells, thymic epithelium
Responsive T cells	CD8$^+$ T cells	CD4$^+$ T cells
Site of antigen degradation	Proteasome	Late endosomes and lysosomes

Feature	**Class I MHC Pathway**	**Class II MHC Pathway**
Source of protein antigens	Mainly cytosolic proteins (usually synthesized in the cell; may enter cytosol from phagosomes); also nuclear and membrane proteins	Endosomal and lysosomal proteins (mostly internalized from extracellular environment)
Enzymes responsible for protein degradation	β1, β2, and β5 subunits of proteasomes	Endosomal and lysosomal proteases (e.g., cathepsins)
Site of peptide loading of MHC	Endoplasmic reticulum	Late endosomes/lysosomes
Molecules involved in transport of peptides and loading of MHC molecules	TAP, tapasin	Invariant chain, DM

APCs, Antigen-presenting cells; *ER*, endoplasmic reticulum; *MHC*, major histocompatibility complex; *TAP*, transporter associated with antigen processing.

- All viruses replicate and survive in infected cells and thus synthesize proteins in the infected cell cytoplasm. These are the most common type of microbial proteins that are processed by the proteasome and presented on class I MHC molecules. The MHC-peptide complexes are then recognized by differentiated, functional CTLs and the infected cells are killed.

- Some bacteria are internalized into phagosomes but are able to damage phagosome membranes and create pores through which the microbes and their antigens enter the cytosol. For instance, pathogenic strains of *Listeria monocytogenes* produce a protein called listeriolysin that enables bacteria to escape from vesicles into the cytosol. (This escape is a mechanism that the bacteria may have developed to resist killing by the microbicidal mechanisms of phagocytes, most of which are concentrated in phagolysosomes.) Once the antigens of the phagocytosed microbes are in the cytosol, they are processed in proteasomes like other cytosolic antigens.
- Some bacteria have type III secretion systems that inject bacterial proteins into the cytosol. Numerous pathogens, including *Yersinia pestis, Salmonella typhi, Shigella dysenteriae, Vibrio cholerae,* and *Chlamydia* species, inject signaling proteins into the host cytosol to manipulate host function and immunity to the pathogen's advantage. This is a major mechanism of bacterial virulence.
- The products of mutated genes in tumors produce antigens in the cytosol of the tumor cells. As in virus-infected cells, display of these tumor antigens on class I MHC molecules enables differentiated CTLs to kill the tumor cells.
- Initiating immune responses against viruses and tumors requires antigen capture by DCs and transport of the antigen-bearing DCs to secondary lymphoid organs, where the antigens can be presented to naive $CD8^+$ T cells. But most viruses infect cells other than DCs, and tumor antigens are produced in the tumor cells, not in DCs. The process by which antigens of other cells (virus-infected or tumor cells) are presented by DCs is called **cross-**

presentation (or **cross-priming**), to indicate that one cell type (the DC) can present antigens from another cell (the virus-infected or tumor cell) and prime, or activate, T cells specific for these antigens. In this process, specialized type 1 conventional DCs (cDC1) and other APCs capture infected or tumor cells or their antigens into vesicles. The vesicles fuse with the endoplasmic reticulum (ER), and by mechanisms that remain poorly defined, proteins from the vesicles are transported into the cytosol.

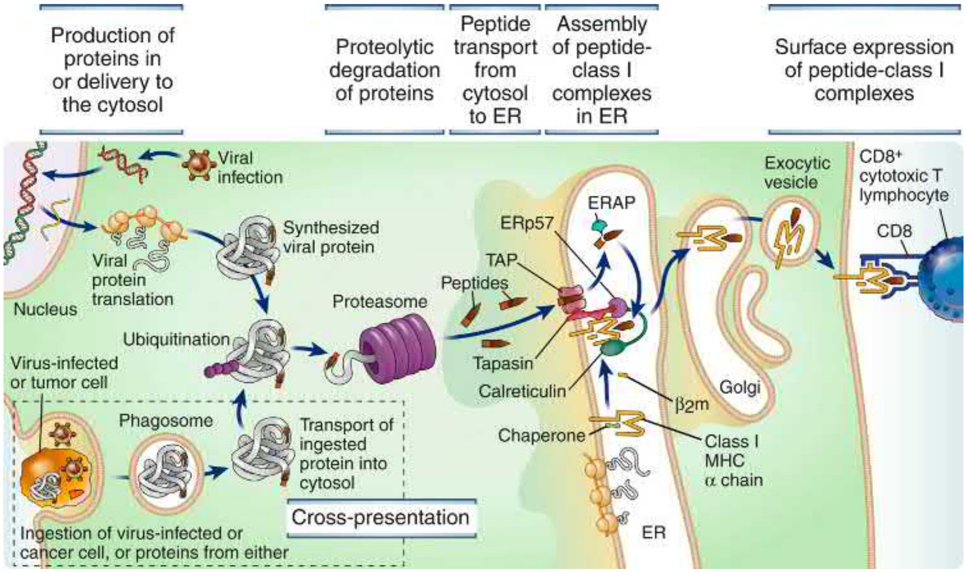

Production of proteins in or delivery to the cytosol
Proteolytic degradation of proteins
Peptide transport from cytosol to ER
Assembly of peptide-class I complexes in ER
Surface expression of peptide-class I complexes
Viral infection
Viral protein translation
Nucleus
Synthesized viral protein
Proteasome
Ubiquitination
Virus-infected or tumor cell
Phagosome
Transport of ingested protein into cytosol
Cross-presentation
Ingestion of virus-infected or cancer cell, or proteins from either
ERp57
ERAP
TAP
Peptides
Tapasin
Calreticulin
Chaperone
β2m
Class I MHC α chain
ER
Golgi
Exocytic vesicle
CD8+ cytotoxic T lymphocyte
CD8

FIGURE 6.14 The class I major histocompatibility complex pathway of antigen presentation.The steps in the processing of cytosolic proteins are described in the text. The proteins may be produced in the cytosol of an infected cell (or a tumor cell, not shown). Other proteins that are ingested into vesicles may be translocated into the cytosol and processed like cytosolic proteins, known as cross-presentation. *β2m,* β2-Microglobulin; *ERAP,* endoplasmic reticulum–associated peptidase; *ER,* endoplasmic reticulum; *TAP,* transporter associated with antigen processing; *Ub,* ubiquitin.

In addition to these microbial antigens, proteins produced in the ER that either do not fold properly or fail to assemble correctly in this compartment are translocated out of the ER and are degraded in proteasomes. Some nuclear proteins also are degraded in proteasomes. These types of proteins are often found in damaged cells and tumors and may be involved in T cell–mediated elimination of these cells.

Digestion of Proteins in Proteasomes

Cytosolic proteins are degraded in proteasomes to generates peptides that are able to bind to class I MHC molecules. Proteasomes are large multiprotein enzyme complexes with a broad range of proteolytic activity that are found in the cytoplasm and nuclei of most cells. A proteasome appears as a cylinder composed of a stacked array of two inner β rings and two outer α rings, each ring being composed of seven subunits, with a caplike structure at each end of the cylinder. The proteins in the outer α rings are structural and lack proteolytic activity; in the inner β rings, three of the seven subunits (β1, β2, and β5) are the catalytic sites for proteolysis.

Proteasomes perform a basic housekeeping function in cells by degrading many damaged or improperly folded proteins. Protein synthesis normally occurs at a rapid rate, with about six to eight amino acid residues being incorporated into elongating polypeptide chains every second. This process is error prone, and it is estimated that approximately 20% of newly synthesized proteins are misfolded. These newly translated but defective polypeptides, as well as proteins that are damaged by cellular stresses, are targeted for proteasomal degradation by covalent linkage of several copies of a small polypeptide called ubiquitin. Proteins with chains of four or more ubiquitins are recognized by the proteasomal cap and then are unfolded, the ubiquitin is removed, and the proteins are threaded through proteasomes, where they are degraded into peptides. The proteasome has broad substrate specificity and can generate a wide variety of peptides from cytosolic proteins (but usually does not degrade them completely into single amino acids).

The composition of proteasomes influences the peptides that are produced. Proteasomes are organelles whose basic cellular function has been adapted for a role in antigen presentation. There are two types of proteasomes with specialized functions in the immune system. **Immunoproteasomes** are present in immune cells, such as DCs and other APCs. They contain three unique catalytic subunits known as β1i, β2i, and β5i in the β ring. The expression of these three subunits is also increased in response to IFNs produced in innate and adaptive immune responses. The production of these subunits results in a change in the substrate specificity of the proteasome such that the peptides produced usually contain carboxy-terminal hydrophobic amino acids such as leucine, valine, isoleucine, and methionine or basic residues such as lysine or arginine. These kinds of C termini are typical of peptides that bind with high affinity to class I molecules. Thus, immunoproteasomes play an important role in generating peptides from foreign proteins that stimulate $CD8^+$ T cells. The second type of proteasome is called the **thymoproteasome** because it is present in thymic epithelial cells. It contains a unique subunit called β5t, which confers upon it the ability to produce peptides that bind weakly to class I MHC molecules. As we will see in Chapter 8, in the thymus these peptides are derived from self proteins, and their low-affinity recognition is important for the process of positive selection, which preserves maturing T cells that strongly recognize foreign antigens. In the absence of the β5t unit (e.g., in mice in which the gene is deleted), $CD8^+$ T cells fail to mature. Predictably, $CD4^+$ T cells are not affected, because, as we discuss later, the peptides that are recognized by $CD4^+$ cells are not generated in the proteasome.

Transport of Peptides From the Cytosol to the Endoplasmic Reticulum

Peptides generated by proteasomes in the cytosol are translocated by a specialized transporter into the ER, where newly synthesized class I MHC molecules are available to bind the peptides. This delivery is mediated by a dimeric protein located in the ER membrane called **transporter associated with antigen processing (TAP),** which is a member of the ABC transporter family of proteins, many of which mediate ATP-dependent transport of low-molecular-weight compounds across cellular membranes. Although the TAP heterodimer has a broad range of specificities, it optimally transports peptides ranging from 8 to 16 amino acids in length and containing carboxyl termini that are basic or hydrophobic. As mentioned earlier, these are the characteristics of the peptides that are generated in the proteasome and are able to bind to class I MHC molecules.

Assembly of Peptide–Class I MHC Complexes in the Endoplasmic Reticulum

Peptides translocated into the ER bind to newly synthesized class I MHC molecules that are associated with the TAP dimer through tapasin. On the luminal side of the ER membrane, the TAP protein associates with a protein called tapasin, which also has an affinity for newly synthesized empty class I MHC molecules. Tapasin is part of a peptide-loading complex, the other proteins in this complex being a thiol oxidoreductase called ERp57 that can break and remake disulfide bonds in proteins and an ER luminal chaperone called calreticulin. Within this complex tapasin forms a stable disulfide-bonded heterodimer with ERp57 and brings the TAP transporter adjacent to the class I MHC molecules that are awaiting the arrival of peptides.

The synthesis and assembly of class I MHC molecules involve a multistep process in which peptide binding plays a key role. Class I α chains and β2-microglobulin are synthesized in the ER. Appropriate folding of the nascent α chains is assisted by chaperone proteins, such as the membrane chaperone calnexin. Within the ER, the newly formed empty class I MHC dimers remain linked to the peptide-loading complex. Peptides that enter the ER through TAP and peptides produced in the ER, such as signal peptides from membrane or secreted proteins, are often trimmed to the appropriate size for MHC binding by the ER-associated aminopeptidase (ERAP). The peptide is then able to bind to the cleft of the adjacent class I MHC molecule. The peptide-loading complex not only delivers peptides to newly synthesized class I MHC molecules but also selects peptides that bind with the highest affinity to class I MHC molecules preferentially over low-affinity-binding peptides. This is a quality control mechanism in antigen processing. Once class I MHC molecules are loaded with peptide, they no longer have an affinity for tapasin, so the peptide-loaded class I MHC molecules are released and are able to exit the ER and be transported to the cell surface. In the absence of bound peptide, many of the newly formed α chain–β2-microglobulin dimers are unstable and cannot be transported efficiently from the ER to the Golgi complex. These misfolded empty class I MHC complexes are transported into the cytosol and eliminated by proteasomal digestion. This process is called ER-associated degradation, but the actual degradation occurs in proteasomes in the cytosol.

Peptides transported into the ER preferentially bind to class I but not class II MHC molecules for two reasons. First, newly synthesized class I MHC molecules are attached to the luminal aspect of the peptide-loading complex, and they capture peptides rapidly as the peptides are transported into the ER by TAP. Second, as discussed later, the peptide-binding clefts of newly synthesized class II molecules in the ER are blocked by a protein called the invariant chain.

Surface Expression of Peptide–Class I MHC Complexes

Class I MHC molecules with bound peptides are structurally stable and are expressed on the cell surface. Stable peptide–class I MHC complexes that were produced in the ER are guided by chaperones to move through the Golgi complex and are transported to the cell surface in exocytic vesicles. Once expressed on the cell surface, the peptide–class I complexes may be recognized by peptide antigen–specific CD8$^+$ T cells, with the CD8 coreceptor playing an essential role by binding to nonpolymorphic regions of the class I MHC molecule. Several viruses and tumors have evolved mechanisms that interfere with class I assembly and peptide loading, emphasizing the importance of this pathway for antiviral and antitumor immunity (see Chapters 16 and 18).

The Class II MHC Pathway for Presentation of Proteins Degraded in Acidic Vesicles

The generation of class II MHC–associated peptides from endocytosed antigens involves the proteolytic degradation of internalized proteins in late endosomes and lysosomes and the binding of peptides to class II MHC molecules in this acidic vesicular compartment. This sequence of events is illustrated in Fig. 6.15, and the individual steps are described next.

Ingestion of Protein Antigens Into Vesicles

Most class II MHC–associated peptides are derived from protein antigens that are ingested into and digested in endosomes and lysosomes in APCs. Proteins that are ingested into vesicles are most commonly extracellular proteins captured by endocytosis, pinocytosis, or phagocytosis, but also include cell surface proteins that are being endocytosed and degraded and intracellular proteins that may be membrane-bound, vesicular, or cytosolic that are included in autophagosomes during the process of autophagy.

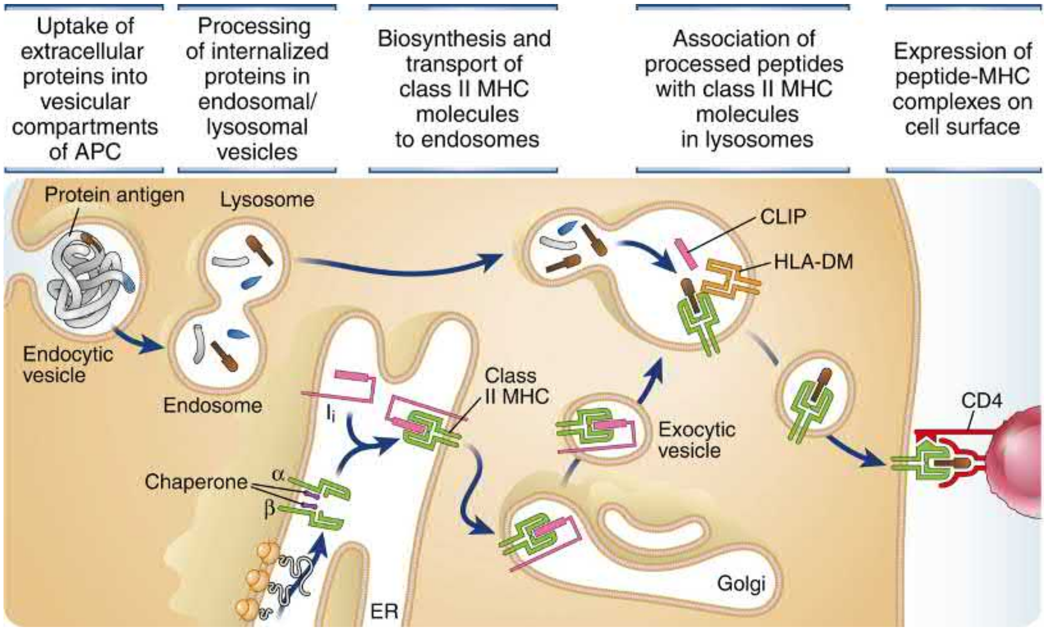

Uptake of extracellular proteins into vesicular compartments of APC
Processing of internalized proteins in endosomal/ lysosomal vesicles
Biosynthesis and transport of class II MHC molecules to endosomes
Association of processed peptides with class II MHC molecules in lysosomes
Expression of peptide-MHC complexes on cell surface
Protein antigen
Lysosome
CLIP
HLA-DM
Endocytic vesicle
Endosome
Class II MHC
Iᵢ
CD4
Exocytic vesicle
Chaperone
α
β
Golgi
ER

FIGURE 6.15 The class II major histocompatibility complex pathway of antigen presentation. The stages in the processing of extracellular antigens are described in the text. *CLIP*, Class II–associated invariant chain peptide; *ER,* endoplasmic reticulum; *HLA,* human leukocyte antigen; I_i, invariant chain.

Different APCs can bind native protein antigens in several ways and with varying efficiencies and specificities.

- DCs and macrophages express a variety of surface receptors, such as lectins, that recognize structures shared by many microbes (see Chapter 4). These APCs use the receptors to bind and internalize microbes efficiently.
- Macrophages also express receptors for the Fc portions of antibodies and receptors for the complement protein C3b, which bind antigens that are opsonized by antibodies or complement proteins and enhance antigen internalization.
- Another example of specific receptors on APCs is the surface Ig on B cells, which, because of its high affinity for antigens, can effectively mediate the internalization of

proteins present at very low concentrations in the extracellular fluid (see Chapter 12).

After the bound protein antigens are internalized, they become localized in intracellular membrane-bound vesicles called endosomes. The endosomal pathway of intracellular protein traffic communicates with lysosomes, which are denser membrane-bound enzyme-containing vesicles. Particulate microbes are internalized into vesicles called phagosomes, which may fuse with lysosomes, producing vesicles called phagolysosomes. Some microbes, such as mycobacteria and *Leishmania,* may survive and even replicate within phagosomes or endosomes, providing a persistent source of antigens in vesicular compartments.

Proteins other than those ingested from the extracellular milieu can also enter the class II MHC pathway.

- Some protein molecules destined for secretion may end up in the same vesicles as class II MHC molecules and may be processed instead of being secreted.
- Cytoplasmic and membrane proteins may be processed and displayed by class II MHC molecules. In some cases, this may result from the enzymatic digestion of cytoplasmic contents, the process known as **autophagy.** In this pathway, cytosolic proteins are trapped within membrane-bound vesicles called autophagosomes, which fuse with lysosomes, and the cytoplasmic proteins are proteolytically degraded. The peptides generated by this route may be delivered to the same vesicular compartment as are peptides derived from ingested antigens. Autophagy is primarily a mechanism for degrading cellular proteins and recycling their products as sources of nutrients during times of stress. It also participates in the destruction of intracellular microbes, which are enclosed in vesicles and delivered to lysosomes.
- Some peptides that associate with class II MHC molecules are derived from membrane proteins that may be recycled

into the same endocytic pathway as are extracellular proteins. Thus, even viruses, which assemble in the cytoplasm of infected cells, may produce proteins that are degraded into peptides that enter the class II MHC pathway of antigen presentation. This may be a mechanism for the activation of viral antigen–specific $CD4^+$ helper T cells.

Proteolytic Digestion of Antigens in Acidic Vesicles

Internalized proteins are degraded enzymatically in late endosomes and lysosomes to generate peptides that are able to bind to the peptide-binding clefts of class II MHC molecules. The degradation of protein antigens in vesicles is mediated by proteases that have acidic pH optima. The most abundant proteases of late endosomes are cathepsins, which are thiol and aspartyl proteases with broad substrate specificities. Several cathepsins contribute to the generation of peptides for the class II pathway. Partially degraded or cleaved proteins bind to the open-ended clefts of class II MHC molecules and are then trimmed enzymatically to their final size.

Biosynthesis and Transport of Class II MHC Molecules to Endosomes

Class II MHC molecules are synthesized in the ER and transported to endosomes with an associated protein, the invariant chain (I_i), which occupies the peptide-binding clefts of the newly synthesized class II MHC molecules (Fig. 6.16) **.** The α and β chains of class II MHC molecules are coordinately synthesized and associate with each other in the ER. The folding and assembly of class II MHC molecules are aided by ER-resident chaperones, such as calnexin. The I_i associates with class II MHC dimers in the ER and directs newly formed class II MHC molecules from the trans-Golgi to late endosomes and lysosomes, where internalized proteins have been proteolytically degraded into peptides. It also prevents the class II molecules from traveling to the cell surface. The I_i is a trimer composed of three 30-kD subunits, each of which binds one newly synthesized class II MHC αβ heterodimer in a way that blocks the peptide-binding cleft and prevents it from accepting peptides. As a result, class II MHC molecules cannot bind and present peptides they encounter in the ER, leaving such peptides to associate with class I MHC molecules (described earlier). The class II MHC molecules are transported in vesicles from the ER to the Golgi. Vesicles budding from the trans-Golgi that contain the class II MHC-I_i complex are transported to lysosomes. Thus, class II MHC molecules encounter antigenic peptides that have been generated by proteolysis of endocytosed proteins in lysosomes, and the peptide-MHC association occurs in these vesicles.

Association of Processed Peptides With Class II MHC Molecules in Vesicles

Within the endosomal/lysosomal vesicles, the I_i dissociates from class II MHC molecules by the combined action of proteolytic enzymes and the HLA-DM molecule, and peptides derived from protein antigens are then able to bind to the available peptide-binding clefts of the class II MHC molecules (see Fig. 6.16) **.** Although class II MHC molecules are relatively resistant to lysosomal proteases, the I_i is degraded in this compartment. The same proteolytic enzymes that generate peptides from internalized proteins, such as cathepsins, also act on the I_i, leaving only a 24 amino acid remnant called class II–associated invariant chain peptide (CLIP), which sits in the peptide-binding cleft. Enzymatic degradation of the transmembrane portion and cytosolic tail of I_i prevents tethering of class II MHC molecules to the lysosomal membrane, and this allows class II MHC–peptide complexes (and some residual MHC class II–CLIP complexes) to bud out of acidic degradatory vesicles and go to the cell surface.

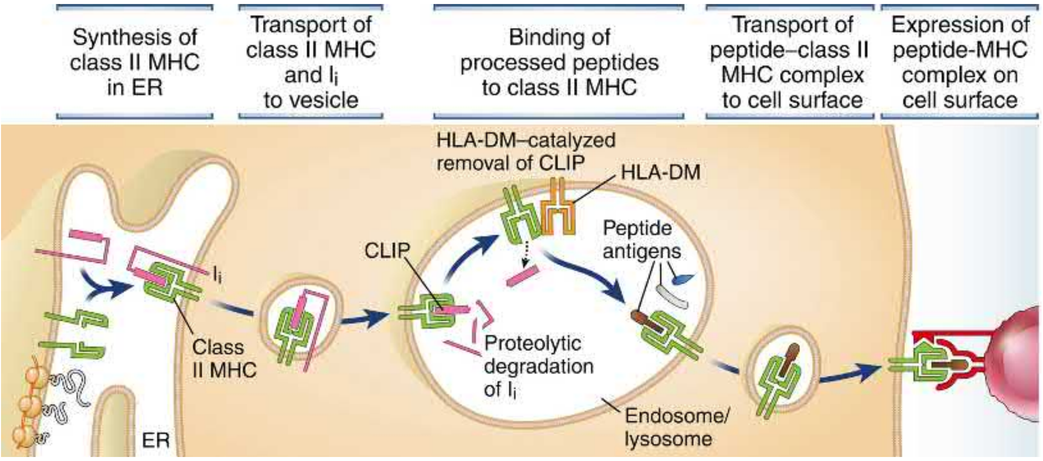

Synthesis of class II MHC in ER
Transport of class II MHC and I_i to vesicle
Binding of processed peptides to class II MHC
Transport of peptide–class II MHC complex to cell surface
Expression of peptide-MHC complex on cell surface
HLA-DM–catalyzed removal of CLIP
HLA-DM
Peptide antigens
CLIP
I_i
Class II MHC
Proteolytic degradation of I_i
Endosome/ lysosome
ER

FIGURE 6.16 The functions of class II major histocompatibility complex–associated invariant chain and human leukocyte antigen DM.Class II MHC molecules with bound invariant chain, or CLIP, are transported into late endosomes and lysosomes, where the I_i is degraded and the remaining CLIP is removed by the action of DM. Antigenic peptides generated in the vesicles are then able to bind to the class II MHC molecules. Another class II–like protein, called DO, may regulate the DM-catalyzed removal of CLIP *(not shown). CIIV,* Class II vesicle; *ER,* endoplasmic reticulum; *I_i* , invariant chain.

The HLA-DM molecule edits the repertoire of peptides being presented, favoring the display of peptides that bind with high affinity to class II MHC molecules. The displacement of CLIP and its replacement by a higher affinity antigenic peptide in lysosomes are accomplished by the action of a molecule called **HLA-DM** (also called DM, or H-2M in the mouse), which is encoded within the MHC, has a structure similar to that of class II MHC molecules, and colocalizes with class II MHC molecules in certain endosomes. Unlike class II MHC molecules, DM molecules are not polymorphic, and they are not expressed on the cell surface. DM binds to the β chain of class II MHC molecules in the region where this chain forms the peptide-binding cleft and dislodges loosely bound peptides from the cleft. It thus acts as a peptide exchanger, facilitating the removal of CLIP and the addition of higher affinity peptides derived from protein antigens to class II MHC molecules. Peptides that bind to the MHC molecules with high affinity cannot be displaced by DM. Thus, the presence of DM is important for selecting peptides that bind strongly to MHC molecules in each individual and displaying these peptides to T cells.

Another dimeric class II MHC-like molecule, called **HLA-DO,** binds to HLA-DM in lysosomes and negatively regulates the function of DM. DM can only mediate peptide exchange after being released from DO. In response to cytokines and other stimuli produced during infections, DM levels rise but DO levels do not, resulting in more efficient peptide exchange and antigen presentation. HLA-DO functions like a chaperone for HLA-DM.

Because the ends of the class II MHC peptide-binding cleft are open, large peptides may bind and are then trimmed by proteolytic enzymes to the appropriate size for T cell recognition. As a result, the peptides that are actually presented attached to cell surface class II MHC molecules are usually 10 to 30 amino acids long and typically have been generated by this trimming step.

Expression of Peptide–Class II MHC Complexes on the Cell Surface

Class II MHC molecules are stabilized by the bound peptides, and the stable peptide–class II complexes are delivered to the surface of the APC, where they are displayed for recognition by CD4^{+} T cells. The transport of class II MHC–peptide complexes to the cell surface is thought to occur by fusion of vesiculotubular extensions from the lysosome to the plasma membrane, resulting in delivery of the loaded class II MHC complexes to the cell surface. Once expressed on the APC surface, the peptide–class II complexes are recognized by peptide antigen–specific CD4^{+} T cells, with the CD4 coreceptor playing an essential role by binding to nonpolymorphic regions of the class II MHC molecule.

Surface class II MHC–peptide levels are regulated by modulation of class II MHC degradation. Class II MHC molecules are normally recycled and degraded by the ubiquitin-proteasome system. A ubiquitin E3 ligase called MARCH-1 recognizes the tail of the class II MHC molecules and targets them for degradation. In response to microbes and cytokines produced during infections, APCs shut off the expression of MARCH-1 and thus increase the amount of the relevant class II–peptide complexes on the cell surface.

Physiologic Significance of MHC–Associated Antigen Presentation

So far, we have discussed the specificity of CD4$^+$ and CD8$^+$ T lymphocytes for MHC-associated foreign protein antigens and the mechanisms by which complexes of peptides and MHC molecules are produced. In this section, we will consider how the central role of the MHC in antigen presentation influences the nature of T cell responses to different antigens and the types of antigens that T cells recognize.

Nature of Effector T Cell Responses

The presentation of cytosolic versus vesicular proteins by the class I or class II MHC pathway, respectively, determines which subset of T cells will recognize antigens found in these two pools of proteins and is intimately linked to the functions of the T cells (Fig. 6.17) . Endogenously synthesized antigens, such as viral and tumor proteins, are located in the cytosol and are recognized by class I MHC–restricted CD8$^+$ CTLs, which kill the cells producing the intracellular antigens. Conversely, extracellular antigens usually end up in endosomal vesicles and activate class II MHC–restricted CD4$^+$ T cells because vesicular proteins are processed into class II–binding peptides. CD4$^+$ T cells function as helpers to stimulate B cells to produce antibodies and activate macrophages to enhance their phagocytic functions, both mechanisms that serve to eliminate extracellular antigens. Thus, antigens from microbes that reside in different cellular locations selectively elicit the T cell responses that are most effective at eliminating that type of microbe. This is especially important because the antigen receptors of CTLs and helper T cells cannot distinguish between extracellular and intracellular microbes. By segregating peptides derived from these types of microbes, the MHC molecules guide $CD4^+$ and $CD8^+$ subsets of T cells to respond to the microbes that each subset can best combat.

Immunogenicity of Protein Antigens

MHC molecules determine the immunogenicity of protein antigens in two related ways.

- ***The epitopes of complex proteins that elicit the strongest T cell responses are the peptides that are generated by proteolysis in APCs and bind most avidly to MHC molecules.*** If an individual is immunized with a protein antigen, in many instances the majority of the responding T cells are specific for only one or a few linear amino acid sequences of the antigen. These are called the immunodominant epitopes or determinants. The proteases involved in antigen processing produce a variety of peptides from natural proteins, and only some of these peptides possess the characteristics that enable them to bind to the MHC molecules present in each individual (Fig. 6.18). It is important to define the structural basis of immunodominance because this may permit the efficient manipulation of the immune system with synthetic peptides. An application of such knowledge is the design of vaccines. For example, a viral protein could be analyzed for the presence of amino acid sequences that would form typical immunodominant epitopes capable of binding to MHC molecules with high affinity. Such analyses can be done experimentally or in silico. Synthetic peptides containing these epitopes may be effective vaccines for eliciting T cell responses against the viral peptides expressed in an infected cell. Similarly, peptides produced by mutated genes in cancers are analyzed for their ability to bind to the class I MHC molecules in each patient with cancer. The ones that bind are most likely to stimulate antitumor immunity in that patient (see Chapter 18).

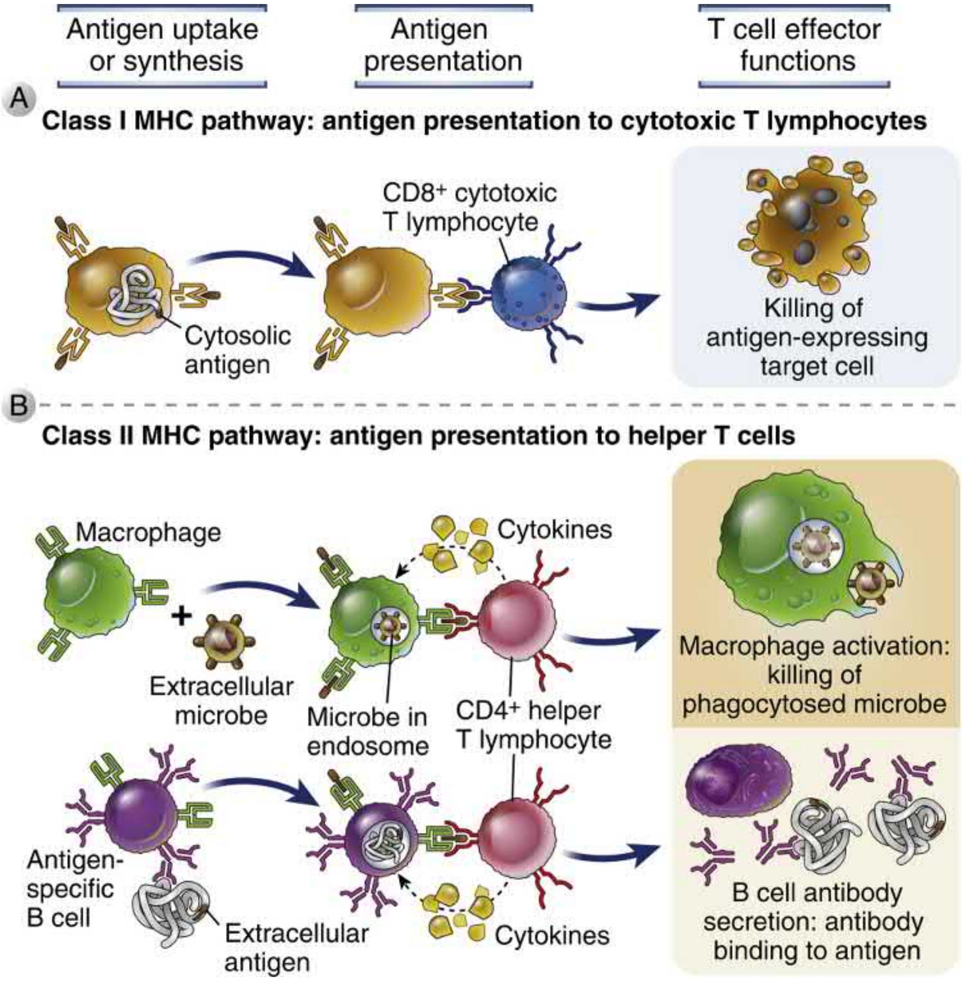

Antigen uptake or synthesis
Antigen presentation
T cell effector functions
A
Class I MHC pathway: antigen presentation to cytotoxic T lymphocytes
CD8+ cytotoxic T lymphocyte
Cytosolic antigen
Killing of antigen-expressing target cell
B
Class II MHC pathway: antigen presentation to helper T cells
Macrophage
Cytokines
+
Extracellular microbe
Microbe in endosome
CD4+ helper T lymphocyte
Macrophage activation: killing of phagocytosed microbe
Antigen-specific B cell
Extracellular antigen
Cytokines
B cell antibody secretion: antibody binding to antigen

FIGURE 6.17 Presentation of extracellular and cytosolic antigens to different subsets of effector T cells. **A,** Cytosolic antigens are presented by nucleated cells to $CD8^+$ cytotoxic T lymphocytes *(CTLs)*, which kill (lyse) the antigen-expressing cells. **B,** Extracellular antigens are presented by macrophages or B lymphocytes to $CD4^+$ helper T lymphocytes, which activate the macrophages or B cells and eliminate the extracellular antigens.

- ***The expression of particular class II MHC alleles in an individual determines the ability of that individual to respond to particular antigens.*** As discussed earlier, the Ir genes that control antibody responses are class II MHC genes. They influence immune responsiveness because various class II MHC molecules produced by different alleles differ in their ability to bind different antigenic peptides and therefore to stimulate specific helper T cells. The consequences of inheriting a given MHC allele depend on the nature of the peptide antigens that can bind the MHC molecule encoded by that allele. For example, if the antigen is a peptide from ragweed pollen, the individual who expresses class II MHC molecules capable of binding the peptide would be genetically prone to allergic reactions against pollen. Conversely, some individuals do not respond to vaccines (such as hepatitis B virus surface antigen vaccine), presumably because their HLA molecules cannot bind and display the major peptides of the vaccine antigen.

Presentation of Nonprotein Antigens to T Cells

T cells also recognize and react against small molecules and even metal ions in an MHC-restricted manner. In fact, exposure to some small molecules that are used as therapeutic drugs and to metals such as nickel and beryllium often leads to pathologic T cell reactions (so-called hypersensitivity reactions; see Chapter 19). There are several ways in which these nonpeptide antigens may be recognized by MHC-restricted $CD4^+$ and $CD8^+$ T cells. Some of the chemicals are thought to covalently modify self peptides or the MHC molecules themselves, creating altered molecules that are recognized as foreign. Other chemicals bind noncovalently to MHC molecules and alter the structure of the peptide-binding cleft such that the MHC molecule can display peptides that are not normally presented, and these peptide-MHC complexes are seen as being foreign.

Several small populations of T cells other than CD4$^+$ and CD8$^+$ cells are able to recognize nonprotein antigens without the involvement of class I or class II MHC molecules. Thus, these populations are exceptions to the rule that T cells can see only MHC-associated peptides. The best defined of these populations are natural killer T (NKT) cells and γδ T cells.

NKT cells express markers that are characteristic of both NK cells and T lymphocytes and express αβ T cell receptors with very limited diversity (see Chapter 10). NKT cells recognize lipids and glycolipids displayed by the class I MHC–like molecule called **CD1.** There are several CD1 proteins expressed in humans and mice. Although their intracellular traffic pathways differ in subtle ways, all CD1 molecules bind and display lipids by a unique mechanism. Newly synthesized CD1 molecules pick up cellular lipids and carry these to the cell surface. From here, the CD1-lipid complexes are internalized into endosomes or lysosomes, where lipids that have been ingested from the external environment are captured and new CD1-lipid complexes are then formed, which are returned to the cell surface. Thus, CD1 molecules acquire endocytosed lipid antigens during recycling and present these antigens without apparent processing. The NKT cells that recognize the lipid antigens may play a role in defense against microbes, especially mycobacteria (which are rich in lipid components).

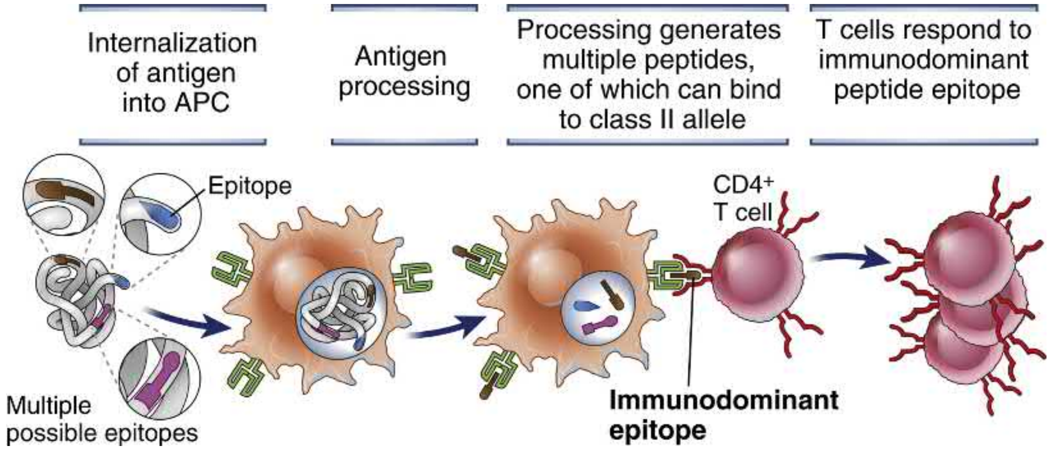

Internalization of antigen into APC
Antigen processing
Processing generates multiple peptides, one of which can bind to class II allele
T cells respond to immunodominant peptide epitope
Epitope
Multiple possible epitopes
CD4+ T cell
Immunodominant epitope

FIGURE 6.18 Immunodominance of peptides.Protein antigens are processed to generate multiple peptides; immunodominant peptides are the ones that bind best to the available class I and class II MHC molecules. The illustration shows an extracellular antigen generating a class II–binding peptide, but this also applies to peptides of cytosolic antigens that are presented by class I MHC molecules. *APC,* Antigen-presenting cell; *MHC,* major histocompatibility complex.

γδ T cells are a small population of T cells that express antigen receptor proteins that are similar but not identical to those of $CD4^+$ and $CD8^+$ T cells (see Chapter 10). γδ T cells recognize many different types of antigens, including some proteins and lipids, as well as small phosphorylated molecules and alkyl amines. These antigens are not displayed by MHC molecules, and γδ cells are not MHC restricted. It is not known if a particular cell type or antigen display system is required for presenting antigens to these cells.

Summary

- The antigen receptors of most T cells recognize only peptides displayed by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells

(APCs). CD4$^+$ helper T lymphocytes recognize antigens in association with class II MHC molecules, and CD8$^+$ CTLs recognize antigens in association with class I MHC molecules.

- APCs capture protein antigens, process them, and display MHC-associated peptides to T cells. Dendritic cells (DCs) are the most efficient APCs for initiating primary responses by activating naive T cells, and macrophages and B lymphocytes present antigens to helper T cells in the effector phase of cell-mediated immunity and in humoral immune responses, respectively. All nucleated cells can present class I–associated peptides, derived from cytosolic proteins, such as viral and tumor antigens, to CD8$^+$ T cells.
- DCs capture antigens from their sites of entry (usually through epithelia) or production (in tissues) and transport these antigens to secondary (peripheral) lymphoid organs. Naive T cells that recirculate through these organs recognize the antigens, and primary immune responses are induced in these organs.
- The MHC is a large genetic region coding for highly polymorphic, codominantly expressed class I and class II MHC molecules.
- Class I MHC molecules are composed of an α (or heavy) chain in a noncovalent complex with a nonpolymorphic polypeptide called β2-microglobulin. Class II MHC molecules contain two MHC-encoded polymorphic chains, an α chain and a β chain. Both classes of MHC molecules consist of an extracellular peptide-binding cleft, a nonpolymorphic immunoglobulin (Ig)-like region, a transmembrane region, and a cytoplasmic region. The peptide-binding cleft of MHC molecules has α-helical sides and an eight-stranded antiparallel β-pleated sheet floor. The polymorphic residues of MHC molecules are localized to the peptide-binding domain.

- The Ig-like domains of class I and class II MHC molecules contain the binding sites for the T cell coreceptors CD8 and CD4, respectively.
- The function of class I and class II MHC molecules is to bind peptide antigens and display them for recognition by antigen-specific T lymphocytes. Peptide antigens associated with class I MHC molecules are recognized by $CD8^+$ T cells, whereas class II MHC–associated peptide antigens are recognized by $CD4^+$ T cells. MHC molecules bind only one peptide at a time. Every MHC molecule has a broad specificity for peptides and can bind multiple peptides that have common structural features, such as anchor residues.
- The peptide-binding cleft of class I MHC molecules can accommodate peptides that are 6 to 16 amino acid residues in length, whereas the cleft of class II MHC molecules allows larger peptides (up to 30 amino acid residues in length or more) to bind. Some polymorphic MHC residues determine the binding specificities for peptides by forming structures called pockets that interact with complementary residues of the bound peptide, called anchor residues. Other polymorphic MHC residues and some residues of the peptide are not involved in peptide binding to MHC molecules but instead form the structure recognized by T cells.
- Class I MHC molecules are expressed on all nucleated cells, whereas class II MHC molecules are expressed mainly on specialized APCs, such as DCs, macrophages, and B lymphocytes, and a few other cell types, including endothelial cells and thymic epithelial cells. The expression of MHC gene products is enhanced by inflammatory and immune stimuli, particularly cytokines such as IFN-γ, which stimulate the transcription of MHC genes.
- Antigen processing is the conversion of native proteins into MHC-associated peptides. This process consists of the introduction of exogenous protein antigens into vesicles of

APCs or the synthesis of antigens in the cytosol, the proteolytic degradation of these proteins into peptides, the binding of peptides to MHC molecules, and the display of the peptide-MHC complexes on the APC surface for recognition by T cells. Thus, both extracellular and intracellular proteins are sampled by these antigen-processing pathways, and peptides derived from both normal self proteins and foreign proteins are displayed by MHC molecules for surveillance by T lymphocytes.

- For the class I MHC pathway, protein antigens are degraded in the proteasome, generating peptides that bind to class I MHC molecules. Most of these antigens are synthesized in the cytosol or introduced into the cytosol from microbes or vesicles. These peptides are delivered from the cytosol to the endoplasmic reticulum (ER) by an ATP-dependent transporter called transporter associated with antigen processing (TAP). Newly synthesized class I MHC–β_2-microglobulin dimers in the ER are associated with the TAP-containing peptide-loading complex and receive peptides transported into the ER. Stable complexes of class I MHC molecules with bound peptides move out of the ER, through the Golgi complex, to the cell surface.
- Specialized APCs, mainly DCs, can ingest virus-infected or tumor cells and transport their antigens into the cytosol for presentation by class I MHC molecules. This process, called cross-presentation, enables DCs to initiate $CD8^+$ T cell responses to the antigens of ingested cells.
- For the class II MHC pathway, protein antigens are internalized into endosomes, and these proteins are proteolytically cleaved by enzymes in lysosomes and late endosomes. Newly synthesized class II MHC molecules associated with the invariant chain (I_i) are transported from the ER to the endosomal vesicles. Here the I_i is proteolytically cleaved, and a small peptide remnant of the I_i, called CLIP, is removed from the peptide-binding cleft of

the MHC molecule by the DM molecules. The peptides that were generated from extracellular proteins then bind to the available cleft of the class II MHC molecule, and the trimeric complex (class II MHC α and β chains and peptide) moves to and is displayed on the surface of the cell.

- These pathways of MHC-restricted antigen presentation ensure that most of the body's cells are screened for the possible presence of foreign antigens. The pathways also ensure that proteins from extracellular microbes preferentially generate peptides bound to class II MHC molecules for recognition by $CD4^+$ helper T cells, which activate effector mechanisms that eliminate extracellular antigens. Conversely, proteins synthesized by intracellular (cytosolic) microbes generate peptides bound to class I MHC molecules for recognition by $CD8^+$ CTLs, which function to eliminate cells harboring intracellular infections. The immunogenicity of foreign protein antigens depends on the ability of antigen-processing pathways to generate peptides from the proteins that bind to self MHC molecules.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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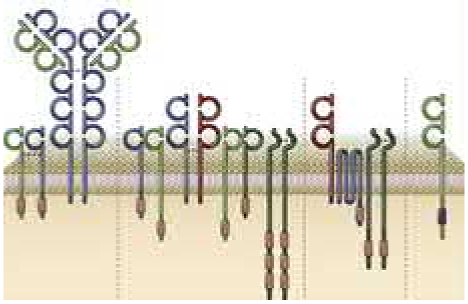
Chapter 7: Immune Receptors and Signal Transduction



- Overview of Signal Transduction,
  - Modular Signaling Proteins and Adaptors,
  - Phase Separation of Signaling Proteins,
- The Immune Receptor Family,
  - General Features of Antigen Receptor Signaling,
- The T Cell Receptor Complex and T Cell Signaling,
  - The T Cell Receptor for Antigen,
  - The Role of the CD4 and CD8 Coreceptors in T Cell Activation,
  - Activation of Tyrosine Kinases and a Lipid Kinase During T Cell Activation,
  - Recruitment and Modification of Adaptor Proteins,
  - Formation of the Immune Synapse,

    - MAP Kinase Signaling Pathways in T Lymphocytes,
    - Calcium- and Protein Kinase C–Mediated Signaling Pathways in T Lymphocytes,
    - Activation of Transcription Factors That Regulate T Cell Gene Expression,
    - Modulation of T Cell Signaling by Protein Tyrosine Phosphatases,
    - Costimulatory Receptor Signaling in T Cells,
    - Metabolic Changes During T Cell Activation,
- The B Lymphocyte Antigen Receptor Complex,
    - Structure of the B Cell Receptor for Antigen,
    - Signal Initiation by the B Cell Receptor,
    - Role of the CR2/CD21 Complement Receptor as a Coreceptor for B Cells,
    - Signaling Pathways Downstream of the B Cell Receptor,
- The Attenuation of Immune Receptor Signaling,
    - Inhibitory Receptors of Natural Killer Cells, B Cells, and T Cells,
    - Ubiquitin-Dependent Degradation of Signaling Proteins,
- Cytokine Receptors and Signaling,
    - Classes of Cytokine Receptors,
    - Signaling by JAKs and STATs,
    - Pathways of NF-κB Activation,
    - TGF-β Signaling,
- Summary,

The idea that cells have specific surface receptors that can be triggered by external ligands came from one of the founders of modern immunology. Paul Ehrlich, in his “side chain theory” published in 1897, conceived of antibodies on the surface of immune cells that recognize antigens and instruct the cells to release more of the same antibody. Cell surface receptors for hormones were discovered many decades later, in the second half of the 20th century, but well before the identification of antigen receptors on lymphocytes in the early 1980s.

Cell surface receptors serve several major functions, including the induction of intracellular signaling leading to cell activation, the adhesion of one cell to another or to the extracellular matrix, and the internalization of extracellular molecules and cells. Signal transduction broadly refers to the intracellular biochemical pathways that are activated in cells after the binding of ligands to specific receptors. Most but not all signaling receptors are located in the plasma membrane. Signaling initiated by these receptors typically involves an initial cytosolic phase when the cytoplasmic portion of the receptor or of proteins that interact with the receptor may be enzymatically modified (Fig. 7.1). This often leads to the activation and/or nuclear translocation of transcription factors that are inactive in resting cells. It is followed by a nuclear phase when the transcription factors bind to target DNA and orchestrate changes in gene expression. Some signal transduction pathways stimulate cell motility or activate granule exocytosis from the cytoplasm without a change in gene expression. Signal transduction can result in a number of different consequences for cells of the immune system, including commitment to a specific lineage, induction of differentiation, protection from cell death, initiation of proliferative responses, performance of effector functions, and induction of cell cycle arrest or of death by apoptosis.

Antigen receptors on B and T lymphocytes are among the most sophisticated cell signaling machines known, and they are discussed in depth in this chapter. We will first provide a broad overview of signal transduction, followed by a discussion of signaling mediated by clonally distributed antigen receptors in lymphocytes. When discussing antigen receptors in T and B cells, we will examine the role of other receptors, including some called coreceptors and others referred to as costimulatory receptors, which enhance lymphocyte activation by the antigen receptor. We will also discuss the role of inhibitory receptors in T, B, and natural killer (NK) cells and consider different categories of cytokine receptors and signal transduction mechanisms initiated by these receptors. Finally, to illustrate the steps in the activation of a prototypic transcription factor, we will examine the pathways that lead to the activation of nuclear factor kappa B (NF-κB), a transcription factor of relevance to both innate and adaptive immunity.

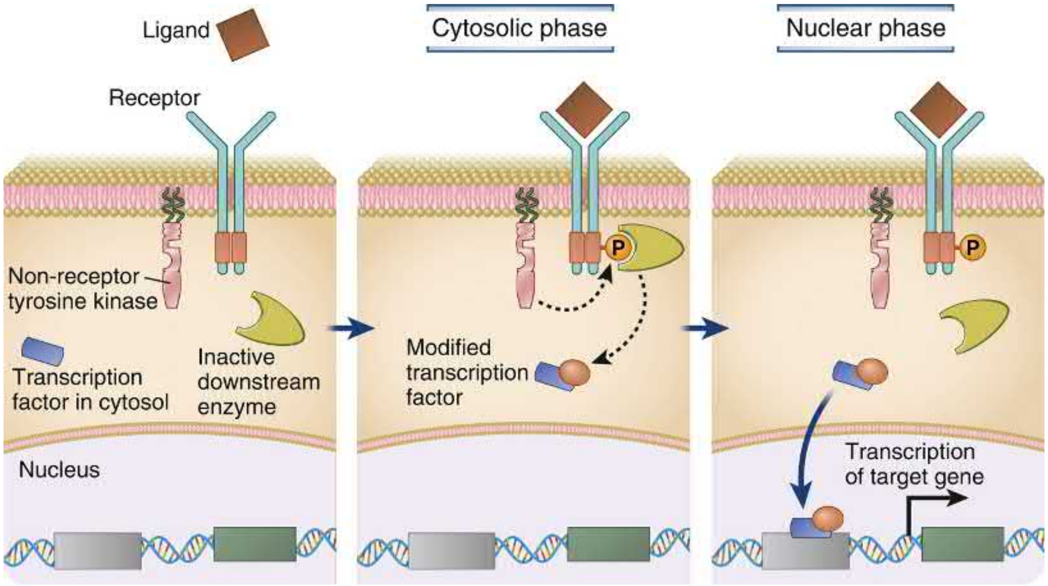

Ligand
Receptor
Cytosolic phase
Nuclear phase
Non-receptor tyrosine kinase
Transcription factor in cytosol
Inactive downstream enzyme
Nucleus
P
Modified transcription factor
P
Transcription of target gene

FIGURE 7.1 Signaling from the cell surface involves cytosolic and nuclear phases.A generic receptor that activates a non-receptor tyrosine kinase after it binds ligand is shown. In the cytosolic signaling phase, the non-receptor kinase phosphorylates a key tyrosine residue on the cytoplasmic tail of the receptor, as a result of which the phosphotyrosine-containing receptor tail is able to recruit a downstream enzyme that is activated once it is recruited. In the cytosolic phase, this activated downstream enzyme post-translationally modifies a specific transcription factor that is located in the cytoplasm. In this simplified example, the cytosolic phase has only a single enzymatic event, but many actual signal transduction pathways involve multiple steps. In the nuclear phase, this modified transcription factor enters the nucleus and binds to a specfic site in the promoter or in some other regulatory region of target genes and thus facilitates their expression.

Overview of Signal Transduction

Receptors that initiate signaling responses are generally integral membrane proteins present on the plasma membrane, where their extracellular domains recognize soluble secreted ligands or structures that are attached to the plasma membrane of a neighboring cell or to the extracellular matrix. Another category of receptors, nuclear receptors, are intracellular (both cytosolic and nuclear family members exist) transcription factors that are activated by lipid-soluble ligands that can cross the plasma membrane.

The initiation of signaling from a cell surface receptor may require ligand-induced clustering of receptor proteins, called cross-linking, or may involve a conformational alteration of the receptor induced by its association with ligand. Both mechanisms of signal initiation typically result in the creation of a structural change in the cytosolic portion of the receptor that promotes the recruitment of and/or interactions with other signaling molecules.

A common early event in signal transduction is the enzymatic addition of a phosphate residue on the side chain of an amino acid in the cytosolic portion of a receptor or in other proteins that are involved in signaling; sometimes a phosphate residue is added to a lipid on the inner leaflet of the plasma membrane. The enzymes that add phosphate groups onto amino acid side chains are called **protein kinases.** Many of the initiating events in lymphocyte signaling depend on protein kinases that phosphorylate specific tyrosine residues, and these enzymes are therefore called protein tyrosine kinases. Other protein kinases that are involved in distinct signaling pathways are serine/threonine kinases, which phosphorylate serine or threonine residues. The enzymes activated downstream of signaling receptors that phosphorylate lipid substrates are known as **lipid kinases.** For most known phosphorylation events, there are also specific phosphatases—enzymes that can remove phosphate residues and thus modulate signaling. These phosphatases play important, usually inhibitory, roles in signal transduction.

Phosphorylation of proteins is not the only post-translational modification that drives signal transduction. Many other modifications can facilitate signaling events. A type of modification that we will describe later in this chapter is the covalent addition of ubiquitin molecules that either target proteins for degradation or drive signal transduction in many cells, including lymphocytes. Many important signaling proteins are modified by the addition of lipids that may help localize these proteins to a specialized region of the plasma membrane in order for them to efficiently interact with other signaling molecules that are also targeted to this membrane microdomain. Some transcription factors are functionally modified by acetylation, and the N-terminal tails of histones can be acetylated and methylated in order to modulate gene expression, DNA replication, and DNA recombination events.

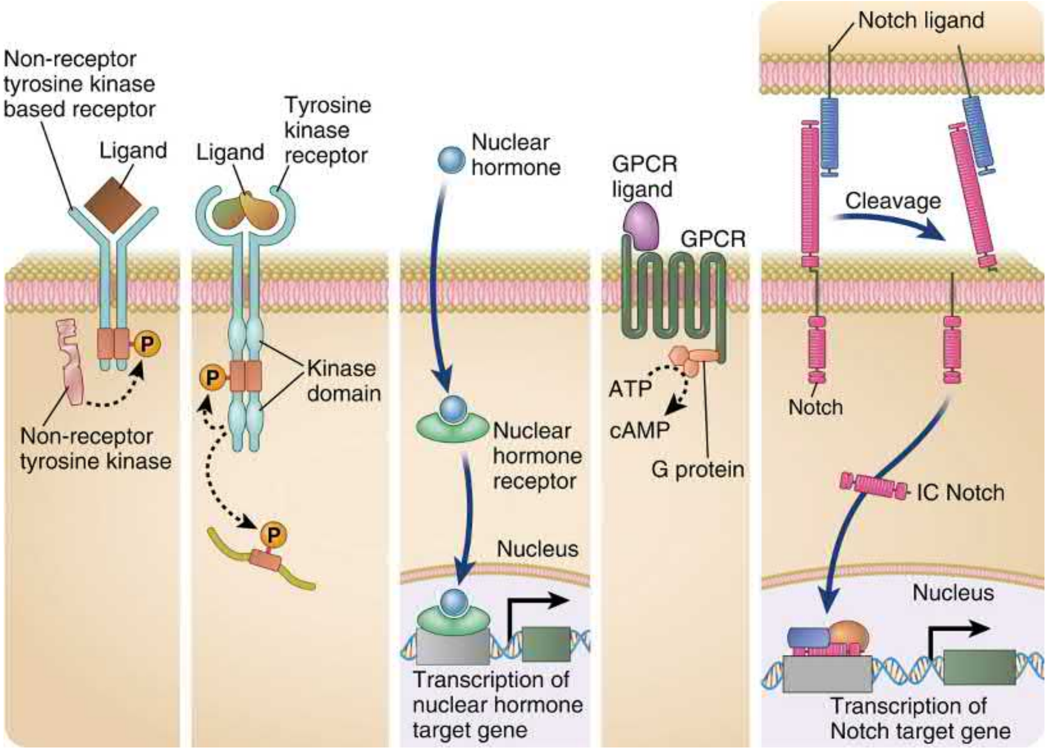

Non-receptor tyrosine kinase based receptor
Ligand
Ligand
Tyrosine kinase receptor
Nuclear hormone
GPCR ligand
GPCR
Notch ligand
Cleavage
P
P
P
Kinase domain
Non-receptor tyrosine kinase
Nuclear hormone receptor
ATP
cAMP
G protein
Notch
IC Notch
Nucleus
Nucleus
Transcription of nuclear hormone target gene
Transcription of Notch target gene

FIGURE 7.2 Major categories of signaling receptors in the immune system.Depicted here are a receptor that uses a non-receptor tyrosine kinase, a receptor tyrosine kinase, a nuclear receptor that binds its ligand and can then influence transcription, a seven-transmembrane G protein–coupled receptor *(GPCR),* and Notch, which recognizes a ligand on a distinct cell and is cleaved, yielding an intracellular fragment *(IC Notch)* that can enter the nucleus and influence transcription of specific target genes. *ATP,* Adenosine triphosphate; *cAMP*, cyclic AMP.

Cellular receptors are grouped into several categories based on the signaling mechanisms they use and the intracellular biochemical pathways they activate (Fig. 7.2) ***:***

- Some receptors use **non-receptor tyrosine kinases.** The cytoplasmic tails of the ligand-binding polypeptides of these receptors have no intrinsic catalytic activity, but a separate intracellular tyrosine kinase, known as a non-receptor tyrosine kinase, participates in receptor activation by phosphorylating specific motifs on the receptor or on other proteins associated with the receptor. Immune receptors constitute a specific family of receptors whose members recognize either antigens or the Fc portions of antibodies; all members of this family use non-receptor tyrosine kinases to initiate signaling. In addition to the immune receptor family, some cytokine receptors, discussed later in this chapter, use non-receptor tyrosine kinases. Integrins, key adhesion receptors in the immune system, may also signal by activating non-receptor tyrosine kinases.

- **Receptor tyrosine kinases (RTKs)** are integral membrane proteins that activate an intrinsic tyrosine kinase domain (or domains) located in the cytoplasmic tails of the receptors when they are cross-linked by multivalent extracellular ligands. This category of receptors is important in hematopoiesis but does not play a central role in lymphocyte activation. An example of an RTK relevant to blood cell formation is the c-KIT protein. Other examples of RTKs include the insulin receptor, the epidermal growth factor receptor, and the platelet-derived growth factor receptor. In vertebrates, a smaller number of receptors have cytosolic catalytic domains that phosphorylate target proteins on serine or threonine residues (and not on tyrosine residues). Two examples will be discussed later in this chapter when we consider transforming growth factor β (TGF-β) signaling.
- **Nuclear receptors** are typically located in or migrate into the nucleus, where they function as transcription factors. The binding of a lipid-soluble ligand to its nuclear receptor enables the receptor to stimulate or repress gene transcription. Nuclear hormone receptors, such as the vitamin D receptor and the glucocorticoid receptor, can

influence maturation and activation of immune cells and modulate cytokine gene expression.

- **G protein–coupled receptors (GPCRs)** function by activating associated guanosine triphosphate (GTP)-binding proteins (G proteins). These receptors are polypeptides that traverse the plasma membrane seven times, because of which they are sometimes called serpentine receptors or seven-transmembrane receptors. A conformational change induced by the binding of ligand to this type of receptor permits the activation of an associated heterotrimeric G protein by the exchange of bound GDP with GTP. The activated G protein initiates downstream signaling events. Examples of this category of receptors that are relevant to immunity and inflammation include receptors for leukotrienes, prostaglandins, histamine, complement fragments C3a and C5a, bacterial formyl peptides, sphingosine-1-phosphate, and all chemokines (see Chapter 3). Different types of G proteins linked to distinct GPCRs may activate or inhibit different downstream effectors. Two major enzymes that GPCRs activate are adenylate cyclase, which converts adenosine triphosphate (ATP) to the effector molecule cyclic adenosine monophosphate (cAMP), capable of activating numerous cellular responses, and phospholipase C, which also triggers multiple signaling events as discussed later.
- **Other classes of receptors** have long been known to be important in embryonic development and in certain mature tissues, and their functions in the immune system have begun to emerge more recently. Receptor proteins of the **Notch** family are involved in development in a wide range of species. The association of specific ligands with receptors of this family leads to proteolytic cleavage of the receptor and the nuclear translocation of the cleaved cytoplasmic domain (intracellular Notch), which functions as a component of a transcription complex. Notch proteins contribute to cell fate determination during lymphocyte

development (see Chapter 8) and may also influence the activation of mature lymphocytes. A group of ligands called **WNT** proteins can influence lymphopoiesis. (The names of many proteins involved in signaling are often based on how they were discovered and do not reflect their functions, so we will use generally accepted abbreviations and not list the full names.) Signaling through transmembrane receptors for these proteins can increase the levels of β-catenin, which can enter the nucleus and activate transcription factors that contribute to B and T cell development, as discussed in Chapter 8. Numerous other signaling receptors and pathways first discovered in non-immune cell populations are now being studied in the context of lymphocyte biology. We will not attempt to comprehensively consider all of these pathways in this chapter.

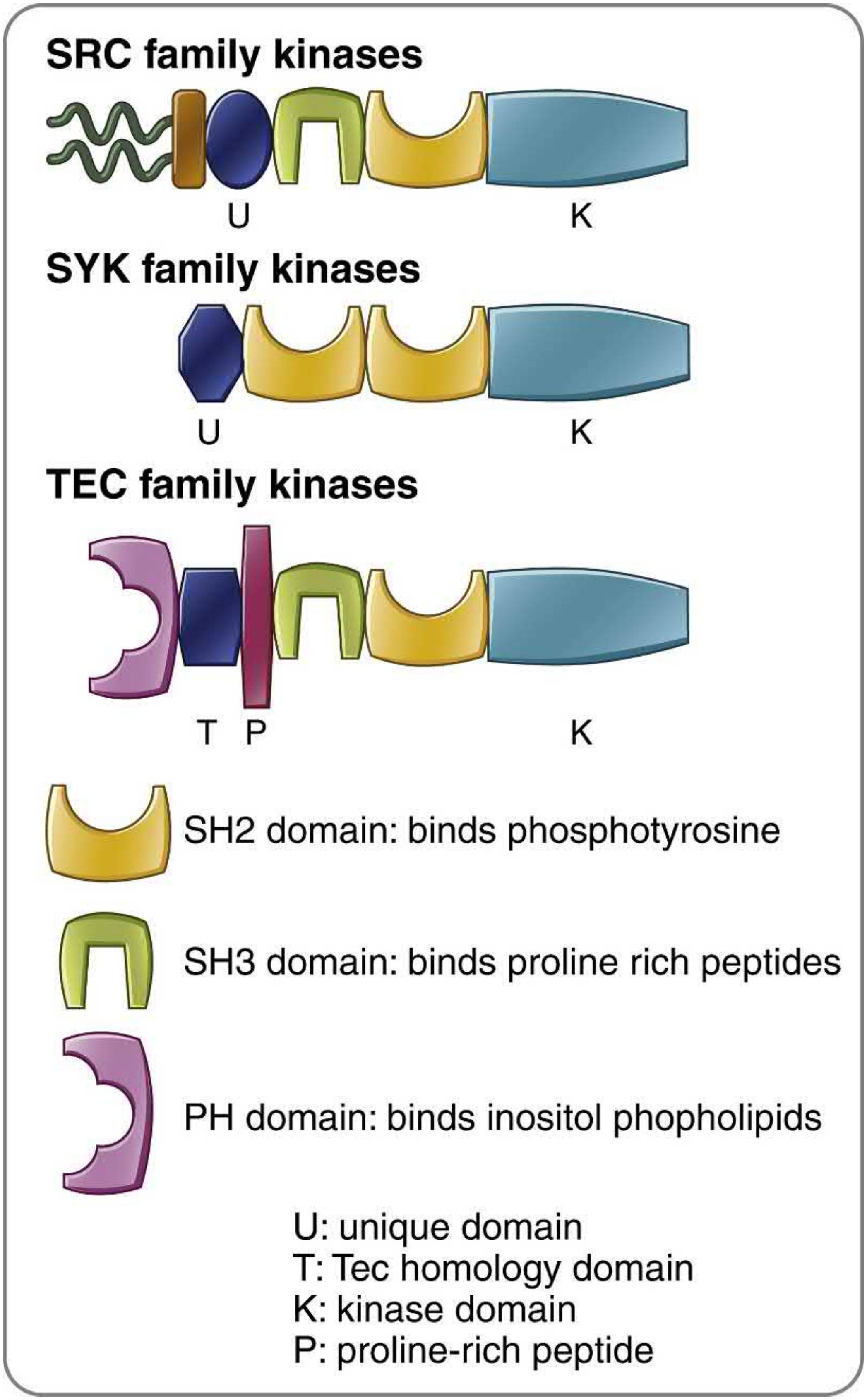

SRC family kinases
U
K
SYK family kinases
U
K
TEC family kinases
T
P
K
SH2 domain: binds phosphotyrosine
SH3 domain: binds proline rich peptides
PH domain: binds inositol phopholipids
U: unique domain
T: Tec homology domain
K: kinase domain
P: proline-rich peptide

FIGURE 7.3 The modular structure of tyrosine kinases that influence lymphocyte activation. Modules include SH2 domains that bind specific phosphotyrosine-containing polypeptides, SH3 domains that recognize proline-rich stretches in polypeptides, PH domains that recognize PIP3 or other phosphatidylinositol-derived lipids, and TEC homology domains found in tyrosine kinases of the TEC family. Tyrosine kinase families depicted are the SRC family kinases, which include c-SRC, LYN, FYN, and LCK; the SYK family kinases, which include SYK and ZAP70; and the TEC family kinases, which include TEK, BTK, and ITK. *PH,* Pleckstrin homology; *SH,* SRC homology.

Modular Signaling Proteins and Adaptors

Signaling molecules are often composed of distinct modules, each with a specific binding or catalytic function. The concept of modular signaling molecules has been best illustrated from the study of non-receptor tyrosine kinases. The modular structures of several families of tyrosine kinases that are important in the immune system are depicted in Fig. 7.3. The cellular homolog of the transforming protein of the Rous sarcoma virus, called c-SRC, is the prototype for an immunologically important family of non-receptor tyrosine kinases known as **SRC family kinases.** c-SRC contains several distinct domains, two of which, called **SRC homology 2** (SH2) and **SRC homology 3** (SH3) domains, mediate binding to other signaling proteins. c-SRC also contains a catalytic tyrosine kinase domain and an N-terminal lipid addition domain that facilitates the covalent addition of a myristic acid molecule to the protein. The myristate helps target SRC family kinases to the plasma membrane.

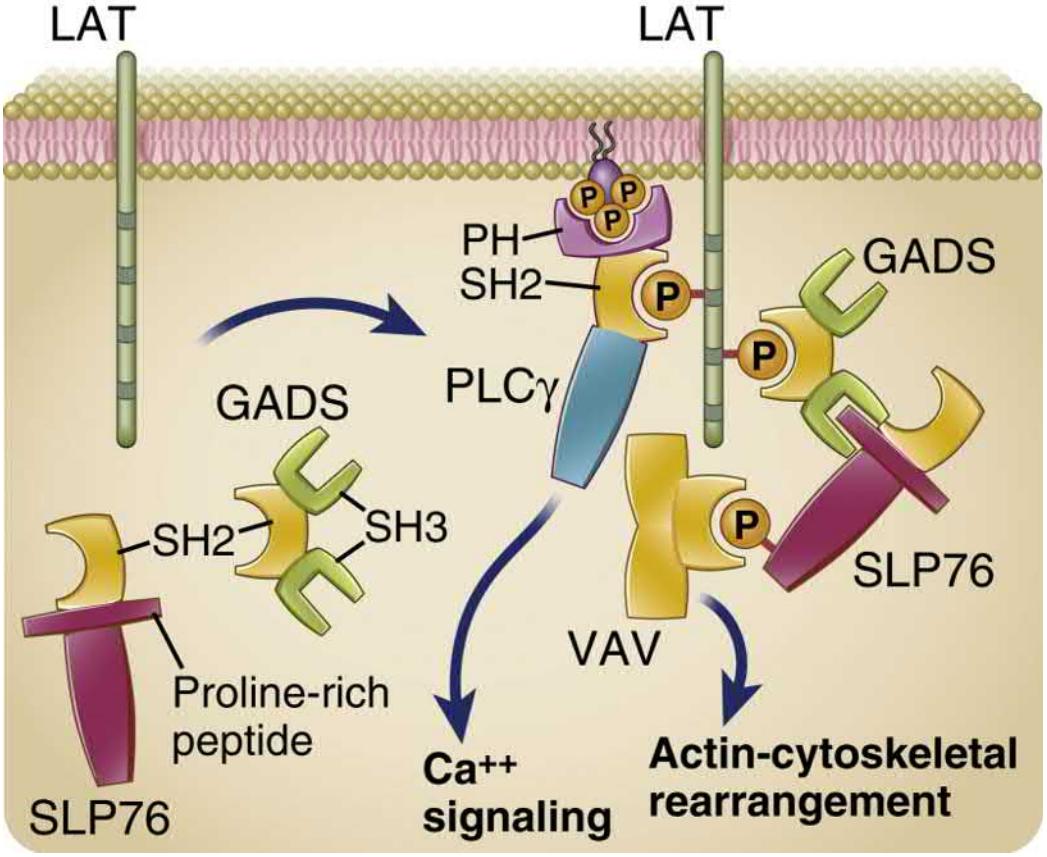

LAT
LAT
PH
SH2
P
P
P
P
P
P
GADS
PLCγ
GADS
SH2
SH3
SLP76
VAV
Proline-rich peptide
SLP76
Ca++ signaling
Actin-cytoskeletal rearrangement

FIGURE 7.4 Selected adaptors that participate in lymphocyte activation.On the *left,* LAT, an integral membrane protein that functions as an adaptor, and two cytosolic adaptors, GADS and SLP76, are shown in a nonactivated T cell. On the *right,* after T cell activation, LAT is tyrosine phosphorylated and is shown to have recruited PLCγ (which simultaneously binds to the membrane phospholipid, phosphatidylinositol trisphosphate, or PIP3) and the GADS adaptor, both of which contain SH2 domains. A proline-rich amino acid stretch in SLP76 associates with an SH3 domain of GADS, and tyrosine-phosphorylated SLP76 recruits VAV. *LAT,* Linker for activation of T cells; *PH,* pleckstrin homology; *PLCγ, phospholipase Cγ; SH, SRC homology.*

SH2 domains are composed of about 100 amino acids folded into a particular conformation, and they bind to phosphotyrosine-containing peptides in various proteins. In antigen receptor signaling, SRC family kinases phosphorylate tyrosine residues present in certain motifs in the cytoplasmic tails of proteins that are part of the receptor complex (described later). These phosphotyrosine motifs in the antigen receptor complex then serve as binding sites for SH2 domains present in tyrosine kinases of the SYK family, namely SYK and ZAP70 (see Fig. 7.3). The recruitment of SYK or ZAP70 to an antigen receptor by means of a specific SH2 domain–phosphotyrosine interaction is a key step in antigen-induced lymphocyte activation. SH3 domains are also about 100 amino acids in length, and they help mediate protein-protein interactions by binding to proline-rich, but not phosphorylated, stretches in certain proteins. Another type of modular domain, called the pleckstrin homology (PH) domain, can recognize specific phospholipids. The PH domains in a number of signaling molecules, including the TEC family tyrosine kinase BTK, recognize phosphatidylinositol trisphosphate (PIP3), a lipid moiety on the inner leaflet of the plasma membrane.

Adaptor proteins function as molecular hubs that physically link different enzymes and promote the assembly of complexes of signaling molecules. Adaptors may be integral membrane proteins such as LAT (Fig. 7.4), or they may be cytosolic proteins such as BLNK, SLP76, and GADS. A typical adaptor may contain a few specific domains that mediate protein-protein interactions, such as those involving SH2 and SH3 domains (there are many more types of modular domains not mentioned here). Adaptors often contain some proline-rich stretches that can bind other proteins that contain SH3 domains, and they also often contain tyrosine residues that may be phosphorylated by tyrosine kinases and serve as docking sites for other signaling molecules. The amino acid residues that are close to a phosphorylated tyrosine moiety determine which specific SH2 domain–containing proteins may bind at that site. For example, a tyrosine kinase may phosphorylate a YxxM motif (where Y represents tyrosine, M represents methionine, and x refers to any amino acid) in an adaptor protein, and this will permit binding of an SH2 domain in the lipid kinase, phosphatidylinositol 3-kinase (PI3-kinase), but will not recruit other proteins that contain slightly different SH2 domains that are not specific for the phosphorylated YxxM motif. A proline-rich stretch in the same adaptor protein may bind an SH3 domain in a distinct tyrosine kinase. Thus, tyrosine phosphorylation of the adaptor can result in a tyrosine kinase and PI3-kinase being perched next to each other, resulting in the phosphorylation and activation of PI3-kinase. Signal transduction can therefore be visualized as a kind of social networking phenomenon. An initial signal (tyrosine phosphorylation, for instance) results in proteins being brought close to one another at designated hubs (adaptors), resulting in the activation of specific enzymes that eventually influence the nuclear localization or activity of specific downstream transcription factors or induce other cellular events, such as actin polymerization.

Phase Separation of Signaling Proteins

An underlying principle of signal transduction is the formation of complexes of activated signaling molecules reorganized into tiny droplets in a different phase of matter, much like the formation of a droplet of oil in water. The phase-separated proteins initiate, amplify, and propagate signals efficiently, primarily because they have been brought into close proximity and at high density. Phase separation is now recognized as a fundamental biologic mechanism wherein distinct activated molecules assemble into a different phase from the neighboring constituents of a cell. This process is important in many reactions, including, but not limited to, signaling and transcription. These include the assembly of adaptors after T cell receptor (TCR) signaling, the activation of cytosolic receptors for nucleic acids and the formation of the inflammasome.

The Immune Receptor Family

Immune receptors are a unique family of receptor complexes typically made up of integral membrane proteins of the immunoglobulin (Ig) superfamily that are involved in ligand recognition, associated with other transmembrane signaling proteins that have unique tyrosine-containing motifs in their cytoplasmic tails (Fig. 7.5). Whereas the signaling components are generally distinct from the proteins involved in ligand recognition, in a few members of the family, the receptor consists of a single chain in which the extracellular domain is involved in ligand recognition and the cytoplasmic tail contains tyrosine residues that contribute to signaling. The signaling proteins of the immune receptor family are often positioned close to non-receptor tyrosine kinases of the SRC family, which possess N-terminal lipid anchors that tether them to the inner leaflet of the plasma membrane.

The cytoplasmic tyrosine-containing motifs on the signaling proteins of the immune receptor family are generally one of three different types, one being an activating motif, the other inhibitory, and the third that can either activate or inhibit depending on the cell type and the particular immune receptor (see Fig. 7.5). **Immunoreceptor tyrosine-based activation motifs (ITAMs)** are found in receptors and associated proteins involved in cell activation and have the sequence YxxL/I$(x)_{6\text{-}8}$YxxL/I, where Y represents a tyrosine residue, L represents leucine, I represents isoleucine, and x refers to any amino acid. Both tyrosine residues in ITAMs can be phosphorylated by SRC family kinases when immune receptors are activated by their ligands. Tyrosine-phosphorylated ITAMs recruit either SYK or ZAP70, which contain tandem SH2 domains that each bind to one of the two phosphorylated YxxL/I motifs of the ITAM. Binding of the SYK or ZAP70 kinase to a phosphorylated ITAM causes a conformational change that activates the kinase, leading to additional signaling events that drive immune cell activation. Some immune receptors inhibit cellular responses, and signaling chains in these receptors may contain a slightly different tyrosine-containing motif that is called an **immunoreceptor tyrosine-based inhibitory motif (ITIM),** which has the consensus sequence V/L/IxYxxL, where V refers to valine. Phosphorylated ITIMs recruit tyrosine phosphatases or inositol lipid phosphatases, enzymes that remove phosphate residues from phosphotyrosine moieties or from certain lipid phosphates and thus counteract ITAM-based immune receptor activation. Certain receptors contain a cytosolic motif called an **immunoreceptor tyrosine-based switch motif (ITSM)** that has the consensus sequence TxYxxV/I. This motif can sometimes function in an inhibitory fashion and recruit an SH2 domain containing tyrosine phosphatase, as is the case with the ITSM in the cytosolic tail of programmed cell death protein-1 (PD-1). It is called a switch motif because in some receptors (of the SLAM family, for instance) this motif can orchestrate a switch from the binding of a tyrosine phosphatase SHP2 to binding a tyrosine kinase, such as FYN, depending on the absence or presence, respectively, of an adaptor called SAP (SLAM-associated protein). Thus, the ITSM can mediate a change from an inhibitory to an activating function.

Members of the immune receptor family include antigen receptors on B cells and T cells, Fc receptors on B cells, myeloid cells and mast cells, and activating and inhibitory receptors on NK cells, T cells, and B cells (see Fig. 7.5) **.** The recognition proteins of several activating receptors in the immune system do not have signaling motifs in their cytoplasmic tails, but signal by forming complexes with ITAM-containing proteins that contribute to signal transduction from the receptor complex. These signaling proteins include the ζ chain and CD3 proteins of the TCR complex, Igα and Igβ proteins associated with the antigen receptor of B cells, and components of several Fc receptors and of the NKG2D activating receptor on NK cells (see Chapter 4). Many inhibitory receptors, including CD22 on B cells, FcγRIIB on B cells and other cells, and several inhibitory NK cell receptors, contain ITIMs in their cytoplasmic domains. The T cell inhibitory receptor PD-1 contains an ITSM motif as well as an ITIM in its tail.

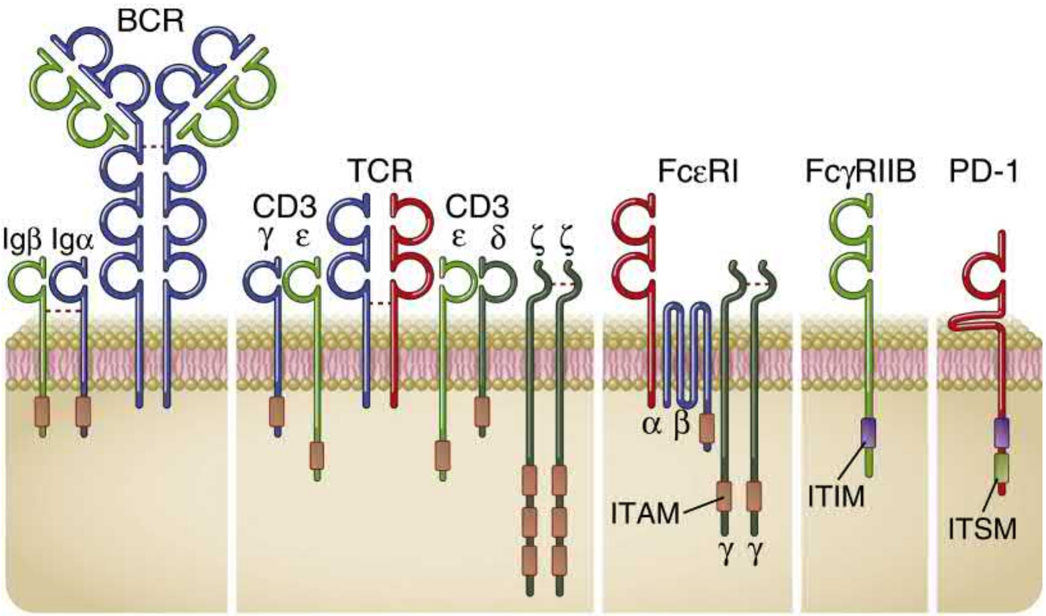


FIGURE 7.5 Selected members of the immune receptor family. Five selected members of the immune receptor family are depicted. Typically, immune receptors that activate immune cells have separate polypeptide chains for recognition and associated polypeptide chains that contain cytosolic ITAMs. Examples shown here include the B cell receptor *(BCR),* the T cell receptor *(TCR),* and the high-affinity receptor for IgE *(FcεRI).* Inhibitory receptors in the immune system typically have ITIMs on the cytosolic portion of the same chain that uses its extracellular domain for ligand recognition. FcγRIIB is an inhibitory receptor found on B cells and myeloid cells. PD-1, an inhibitory receptor found on T cells, also has an immunoreceptor tyrosine-based switch motif *(ITSM)* in its cytoplasmic tail. *Ig,* Immunoglobulin; *ITAM,* immunoreceptor tyrosine-based activation motif; *ITIM,* immunoreceptor tyrosine-based inhibition motif.

General Features of Antigen Receptor Signaling

Signaling downstream of T and B cell antigen receptors is characterized by a similar sequence of events, consisting of the following:

- Receptor ligation typically involves the clustering of receptors by multivalent ligands and results in activation of an associated SRC family kinase. In some cases, a SRC kinase is brought into proximity with the receptor and associated proteins. Receptor ligation may also induce the unfolding of the cytoplasmic tail of a polypeptide chain that is part of the receptor. This conformational change may allow previously hidden tyrosine residues of a cytosolic ITAM to become available for phosphorylation by the SRC family kinase.
- The activated SRC family kinase phosphorylates available tyrosines in the ITAMs of signaling proteins that are part of the receptor complex.
- Two phosphorylated tyrosines in a single ITAM are recognized by SYK or ZAP70 kinases, which contain tandem SH2 domains that each bind to an ITAM phosphotyrosine.
- Recruitment of SYK or ZAP70 to the phosphorylated ITAM results in the activation of the kinase and the subsequent tyrosine phosphorylation of multiple adaptor proteins and enzymes that activate distinct signaling pathways downstream of the immune receptor.

This sequence of events is described in more detail in the context of TCR and B cell receptor (BCR) signaling later in the chapter.

Alterations in the strength of TCR and BCR signaling influence the responses of lymphocytes during their development and activation. In other words, the presence of different numbers of activated signaling molecules induced by antigen-ligated receptors is interpreted differently by lymphocytes. For example, during lymphocyte development, weak antigen receptor signaling is required for survival of clones expressing functional receptors (positive selection), and strong signaling is required to induce apoptosis of clones with self-reactive antigen receptors (negative selection). In mature lymphocytes, strong signaling usually results in clonal expansion and differentiation of naive lymphocytes, and the acquisition of host defense–related functions by effector lymphocytes.

Antigen receptor signaling is modulated by mechanisms that are unique to this class of receptors. Several such mechanisms have been described.

- ***Progressive ITAM use.*** One of the ways in which the quantity of signal output from antigen receptors might be altered is the phosphorylation of different numbers of ITAM tyrosines after receptor engagement. The TCR complex has 6 signaling chains and 10 ITAMs, and increasing numbers of ITAMs may be phosphorylated with stronger or prolonged binding of antigen to the TCR. The number of ITAMs phosphorylated may therefore provide a cytosolic interpretation of the strength of antigen binding to the TCR, and this can influence the nature of the cellular response. The BCR has only two ITAMs, but because this number increases when multiple BCRs are cross-linked by multivalent antigens, the degree of cross-linking by antigens may determine the number of ITAMs that might be used and thus generate different responses to antigens of differing affinity and valency.
- ***Increased cellular activation by coreceptors.*** A **co-receptor** is a transmembrane signaling protein on a lymphocyte that can facilitate antigen receptor activation by simultaneously binding to the same antigen complex that is recognized by the antigen receptor. The coreceptor brings with it signaling enzymes linked to its cytoplasmic tail and can thereby facilitate ITAM phosphorylation and activation of the

antigen receptor when antigen draws it into the vicinity of the antigen receptor. Coreceptors on T cells are the CD4 and CD8 proteins that demarcate two functionally distinct subsets. Complement receptor type 2 (CR2/CD21) is the coreceptor on B cells (see Chapter 12).

- ***Modulation of signaling by inhibitory receptors.*** Key **inhibitory receptors** in T cells include CTLA-4 and PD-1, and important inhibitory receptors in B cells are CD22 and FcγRIIB, among others. The roles of these inhibitors are discussed later in this chapter.

In addition, antigen receptor signals may, in some circumstances, cooperate with signals from proteins called **costimulatory receptors** that add yet another level of control to the process of lymphocyte activation. This process is best documented for T cell activation. Costimulatory receptors provide so-called second signals for lymphocytes (antigen recognition provides the first signal) and ensure that immune responses are optimally triggered by infectious pathogens and substances that mimic microbes, which are the agents that induce or activate costimulators (see Figs. 4.19 and 9.3). Unlike coreceptors, costimulatory receptors do not bind to the antigens that are recognized by the antigen receptor, but rather to entirely distinct ligands on an antigen-presenting cell (APC) that are induced by pathogens and other potentially dangerous insults. Signal outputs downstream of costimulatory receptors cooperate with the signals derived from the antigen receptor to fully activate lymphocytes. The prototypic costimulatory receptor is CD28 on T cells, which is activated when bound by the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on APCs (see Chapter 9).

This discussion of the general principles of signaling in the immune system provides the basis for considering in more detail the specific pathways activated in T and B cells by receptors for antigen and other stimulators.

The T Cell Receptor Complex and T Cell Signaling

The TCR was discovered in the early 1980s, at around the same time that the structure of major histocompatibility complex (MHC) molecules with bound peptides, the ligands for T cells, was being defined (see Chapter 6). This was years after the B cell antigen receptor and Ig genes were characterized. The methods used to search for the proteins of the TCR and the genes encoding them relied on the assumption that they would be similar to Ig proteins and genes. We now know that TCRs are similar to antibodies, but there are also important differences between these two types of antigen receptors (Table 7.1).

TABLE 7.1

	T Cell Receptor (TCR)	Immunoglobulin (Ig)
Components	α and β chains (most common form of TCR)	Heavy and light chains
Number of Ig domains	One V domain and one C domain in each chain	Heavy chain: One V domain, three or four C domains Light chain: One V domain and one C domain
Number of CDRs involved in antigen binding	Six (three in each chain)	Six (three in each chain)
Associated signaling molecules	CD3 and ζ	Igα and Igβ
Affinity for antigen (K_d)	10^{-5}–10^{-7} M	10^{-7}–10^{-11} M
Changes After Cellular Activation		
Production of secreted form	No	Yes
Isotype switching	No	Yes
Somatic mutations	No	Yes

CDRs, Complementarity-determining regions.

The T Cell Receptor for Antigen

The antigen receptor of MHC-restricted CD4$^+$ helper T cells and CD8$^+$ cytotoxic T lymphocytes (CTLs) is a heterodimer consisting of two transmembrane polypeptide chains, designated TCR α and β, covalently linked to each other by a disulfide bridge between extracellular cysteine residues (Fig. 7.6) **.** T cells expressing this form of TCR are called αβ T cells. A less common type of TCR is composed of TCR γ and δ chains, and the cells on which it is expressed are called γδ T cells. Each TCR α and β chain consists of one Ig-like N-terminal variable (V) domain, one Ig-like constant (C) domain, a hydrophobic transmembrane region, and a short cytoplasmic region. Thus, the extracellular portion of the TCR αβ heterodimer is structurally similar to the antigen-binding fragment (Fab) of an Ig molecule, which is made up of the V and C regions of a light chain and the V region and the first C region of a heavy chain (see Chapter 5).

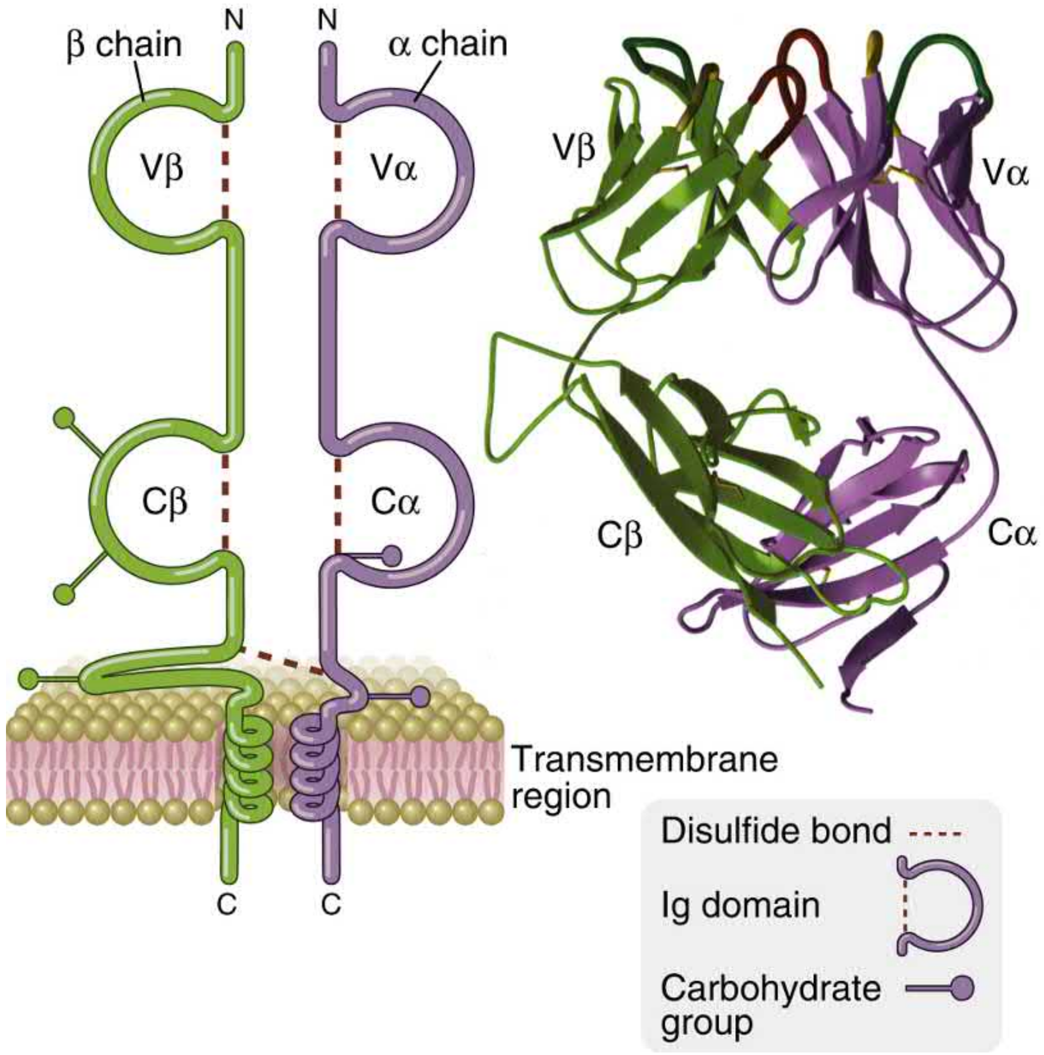

β chain
N
N
α chain
Vβ
Vα
Cβ
Cα
C
C
Transmembrane region
Vβ
Vα
Cβ
Cα
Disulfide bond
Ig domain
Carbohydrate group

FIGURE 7.6 Structure of the T cell receptor. The schematic diagram of the αβ T cell receptor (TCR) *(left)* shows the domains of a typical TCR specific for a peptide–major histocompatibility complex (MHC) complex. The antigen-binding portion of the TCR is formed by the Vβ and Vα domains. The ribbon diagram *(right)* shows the structure of the extracellular portion of a TCR as revealed by x-ray crystallography. The hypervariable segment loops that form the peptide-MHC binding site are at the top. *Ig,* Immunoglobulin.

Modified from Bjorkman PJ. MHC restriction in three dimensions: a view of T cell receptor/ligand interactions. *Cell.* 1997;89:167–170. Copyright Cell Press.

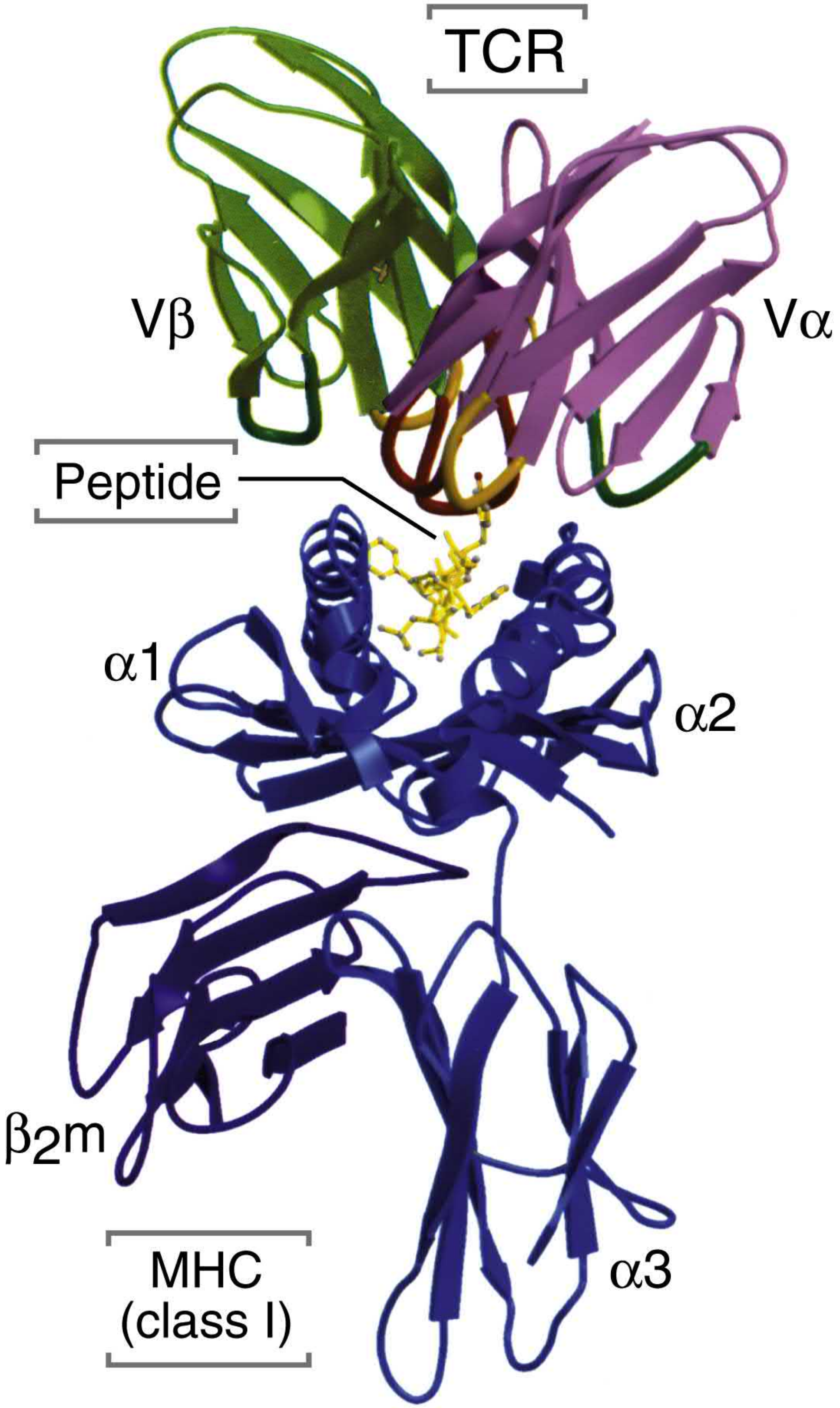

TCR
Vβ
Vα
Peptide
α1
α2
β2m
MHC (class I)
α3

FIGURE 7.7 Binding of a T cell receptor to a peptide–major histocompatibility complex.The V domains of a TCR are shown interacting with a human class I MHC molecule, HLA-A2, presenting a viral peptide *(in yellow)*. The figure represents a front view of the x-ray crystal structure of the trimolecular MHC-peptide–TCR complex. *β2m,* Beta-2 microglobulin; *HLA,* human leukocyte antigen; *MHC,* major histocompatibility complex; *TCR,* T cell receptor.

From Bjorkman PJ. MHC restriction in three dimensions: a view of T cell receptor/ligand interactions. *Cell.* 1997;89:167–170. Copyright Cell Press.

The V regions of the TCR α and β chains contain short stretches of amino acids where the variability between different TCRs is concentrated, and these form the hypervariable or complementarity-determining regions (CDRs). Three CDRs in the α chain and three similar regions in the β chain together form the part of the TCR that specifically recognizes peptide-MHC complexes (Fig. 7.7). Each TCR chain, like Ig heavy and light chains, is encoded by multiple gene segments that are joined together during the maturation of T lymphocytes (see Chapter 8).

The C regions of both α and β chains continue into short hinge regions, which contain cysteine residues that contribute to a disulfide bond linking the two chains. Each hinge is followed by a hydrophobic transmembrane portion and carboxy-terminal cytoplasmic tails that are 5 to 12 amino acids long. Like membrane IgM on B cells (discussed later), the cytoplasmic regions are too short to transduce signals, and other proteins with ITAM motifs that are physically associated with the TCR serve the signal-transducing functions of this antigen receptor complex.

The CD3 and ζ proteins are noncovalently associated with the TCR αβ heterodimer to form the TCR complex, and when the TCR recognizes antigen, these associated proteins transduce the signals that lead to T cell activation. The components of the TCR complex are illustrated in Fig. 7.8. The CD3 proteins and the ζ chain are identical in all T cells regardless of specificity, which is consistent with their role in signaling and not in antigen recognition. The CD3 and ζ proteins are also required for surface expression of the complete receptor complex on T cells.

The CD3 γ, δ, and ε proteins are homologous to one another. The N-terminal extracellular regions of the γ, δ, and ε chains of CD3 each contains a single Ig-like domain, and therefore these three proteins are members of the Ig superfamily. The transmembrane segments of all three CD3 chains and the ζ chain contain a negatively charged aspartic acid residue that binds to positively charged lysine and arginine residues in the transmembrane domains of the TCR α and β chains, thus keeping the TCR, CD3, and ζ associated as a complex. Each TCR complex contains one TCR αβ heterodimer associated with one CD3 γε heterodimer, one CD3 δε heterodimer, and one disulfide-linked ζζ homodimer.

The cytoplasmic domains of the CD3 γ, δ, and ε proteins range from 44 to 81 amino acid residues in length, and each of these domains contains one ITAM. The ζ chain has a short extracellular region of nine amino acids, a transmembrane region containing a negatively charged aspartic acid residue (similar to the CD3 chains), and a long cytoplasmic region (113 amino acids) that contains three ITAMs. The ζ chain is normally expressed as a homodimer, and it is also associated with signaling receptors on lymphocytes other than T cells, such as the Fcγ receptor (FcγRIII) of NK cells.

Ligation of the TCR by MHC-peptide ligands results in the clustering of coreceptors with the antigen receptor and phosphorylation of ITAM tyrosine residues in CD3 and ζ proteins. In addition, recognition of peptide-MHC complexes by the TCR may induce a conformational change in the TCR, making the ITAMs associated with the linked CD3 or ζ chains available for tyrosine phosphorylation by coreceptor-associated SRC family kinases.

In addition to the TCR complex, T cells express several other proteins that recognize ligands on APCs and play important roles in T cell responses (Fig. 7.9).

The Role of the CD4 and CD8 Coreceptors in T Cell Activation

CD4 and CD8 are T cell coreceptors that bind to nonpolymorphic regions of MHC molecules and facilitate signaling by the TCR complex during T cell activation (see Fig 7.9)**.** Mature αβ T cells express either CD4 or CD8 but not both. CD8 and CD4 interact with class I and class II MHC molecules, respectively, and are responsible for the class I or class II MHC restriction of these classes of T cells (see Chapter 6).

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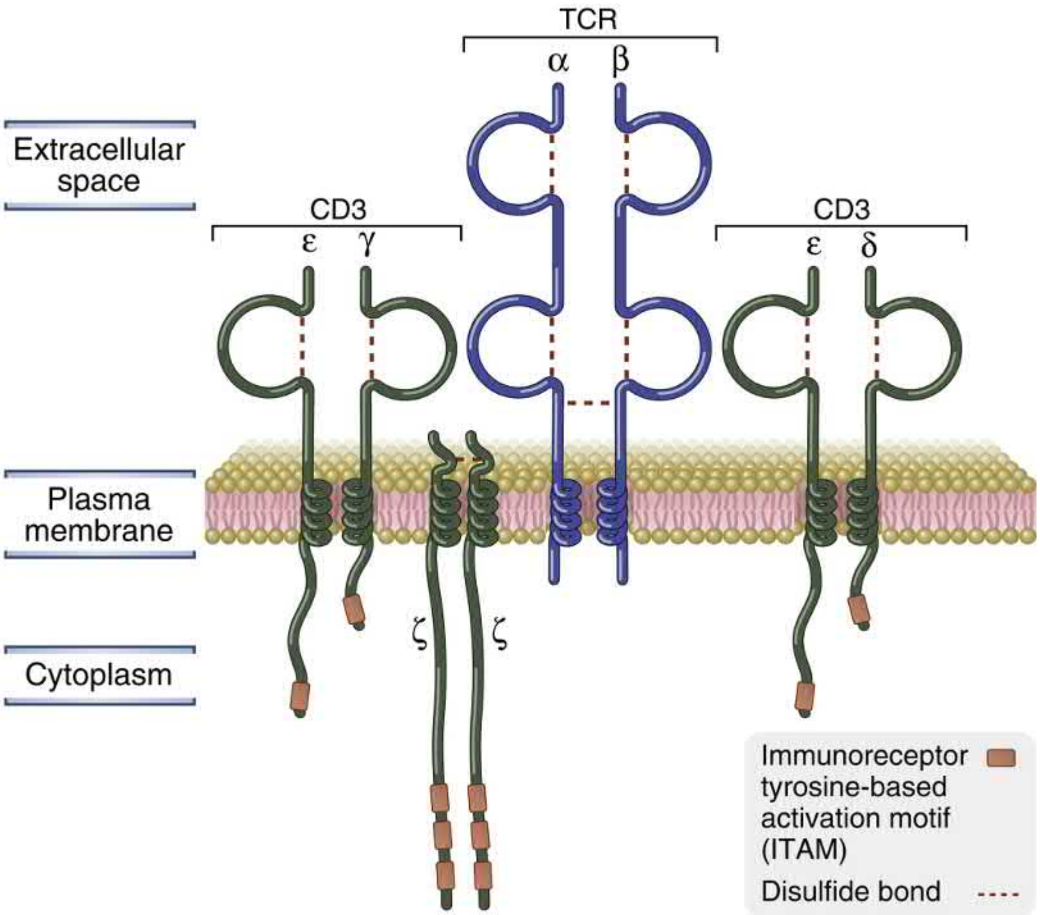
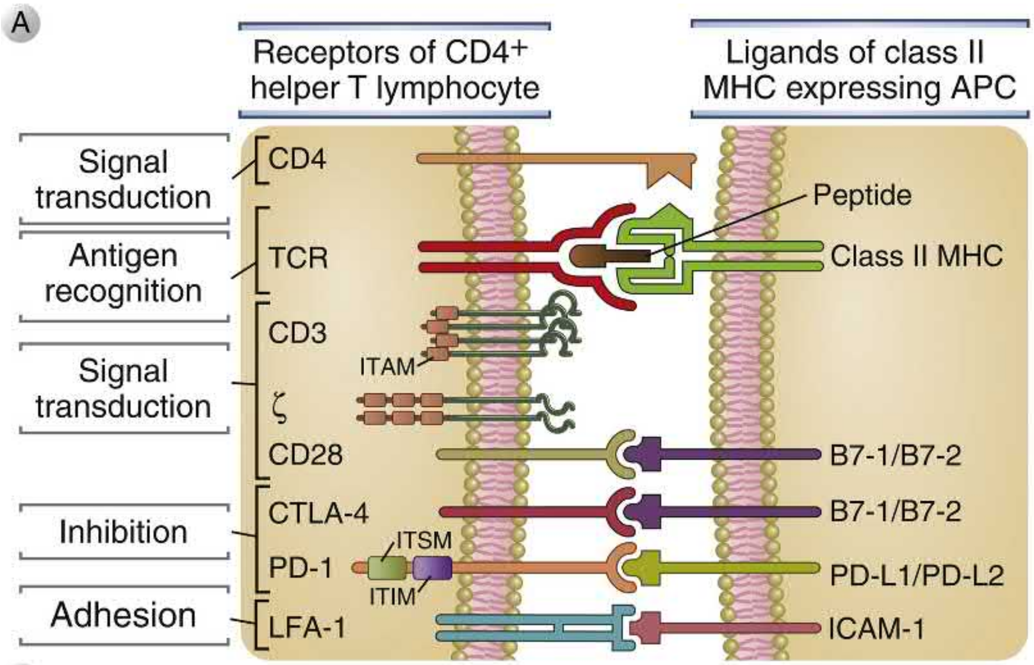

TCR
α
β
Extracellular space
CD3
ε
γ
CD3
ε
δ
Plasma membrane
Cytoplasm
ζ
ζ
Immunoreceptor tyrosine-based activation motif (ITAM)
Disulfide bond

FIGURE 7.8 Components of the T cell receptor complex. The T cell receptor *(TCR)* complex of major histocompatibility complex–restricted T cells consists of the αβ TCR noncovalently linked to the CD3 and ζ proteins. The association of these proteins with one another is mediated by charged residues in their transmembrane regions *(not shown)*.

CD4 and CD8 are transmembrane glycoprotein members of the Ig superfamily (Fig. 7.10). CD4 is expressed as a monomer on the surface of peripheral T cells and thymocytes and is also present at lower levels on mononuclear phagocytes and some dendritic cells (DCs). CD4 has four extracellular Ig-like domains, a hydrophobic transmembrane region, and a highly basic cytoplasmic tail 38 amino acids long. The two N-terminal Ig-like domains of the CD4 protein bind to the nonpolymorphic α2 and β2 domains of the class II MHC molecule. The human immunodeficiency virus (HIV) uses CD4 as a receptor to gain entry into T lymphocytes and other immune cells that express the molecule (see Chapter 21).

Most CD8 molecules exist as disulfide-linked heterodimers composed of two related chains called CD8α and CD8β (see Fig. 7.10). Both the α and β chains have a single extracellular Ig domain, a hydrophobic transmembrane region, and a highly basic cytoplasmic tail that is about 25 amino acids long. Activated T cells as well as γδ T cells can express CD8αα homodimers. The Ig domains of CD8 bind mainly to the nonpolymorphic α3 domain of class I MHC molecules, and also interact with portions of the α2 domain and with β2 microglobulin.

The SRC family kinase LCK is noncovalently bound to the cytoplasmic tails of the coreceptors CD4 and CD8. The ability of the extracellular domains of these coreceptors to bind to MHC molecules on an APC draws these proteins adjacent to the TCR that contacts the same MHC molecule that is displaying a peptide on the APC. As a result, on the cytosolic face of the plasma membrane, LCK is brought in close proximity to the ITAMs in CD3 and ζ proteins. LCK then phosphorylates the tyrosine residues in these ITAMs, thus facilitating the subsequent recruitment and activation of the ZAP70 tyrosine kinase. Note that LCK is already attached to both the CD4 and CD8 coreceptors and is active even before antigen exposure; the other proteins in the TCR complex, CD3 and ζ, contain ITAMs that first need to be phosphorylated before they can recruit a kinase. Thus, the coreceptor provides the earliest enzymatic activity for initiating signals after T cell recognition of peptide-MHC complexes.


A
Receptors of CD4+ helper T lymphocyte
Ligands of class II MHC expressing APC
Signal transduction
Antigen recognition
Signal transduction
Inhibition
Adhesion
CD4
TCR
CD3
ITAM
ζ
CD28
CTLA-4
ITSM
PD-1
ITIM
LFA-1
Peptide
Class II MHC
B7-1/B7-2
B7-1/B7-2
PD-L1/PD-L2
ICAM-1

B

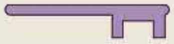
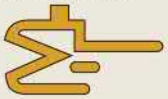
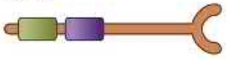
T cell molecule	**Function**	**Ligand**	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8 	Signal transduction	Class I MHC 	All nucleated cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Inhibition	B7-1/B7-2	Antigen presenting cells
PD-1 	Inhibition	PD-L1/PD-L2	Antigen presenting cells, tissue cells, tumor cells
LFA-1	Adhesion	ICAM-1	Antigen presenting cells, endothelium

FIGURE 7.9 Ligand-receptor pairs involved in T cell activation. **A,** The major surface molecules of CD4$^+$ T cells involved in the responses of these cells (the receptors) and the molecules on APCs (the ligands) recognized by the receptors are shown. CD8$^+$ T cells use most of the same molecules, except that the TCR recognizes peptide–class I MHC complexes, and the coreceptor is CD8, which recognizes class I MHC. Immunoreceptor tyrosine-based activation motifs *(ITAMs)* are the regions of signaling proteins that are phosphorylated on tyrosine residues and become docking sites for other signaling molecules. CD3 is composed of three polypeptide chains, named γ, δ, and ε, arranged in two pairs (γε and δε) as shown in Fig. 7.8. Some inhibitory receptors such as PD-1 contain cytoplasmic immunoreceptor tyrosine-based inhibitory motifs *(ITIMs)* and "switch" motifs (immunoreceptor tyrosine-based switch motifs [ITSMs]). **B,** Important molecules of T cells that participate in activating or inhibiting responses to antigens, but are not the receptors for antigen, are summarized. *APCs,* Antigen-presenting cells; *CTLA-4,* cytotoxic T lymphocyte antigen-4; *ICAM-1,* intercellular adhesion molecule 1; *LFA-1,* leukocyte function–associated antigen 1; *MHC,* major histocompatibility complex; *PD-1,* programmed cell death protein-1; *PDL-1/2,* programmed death ligands 1 and 2; *TCR,* T-cell receptor.

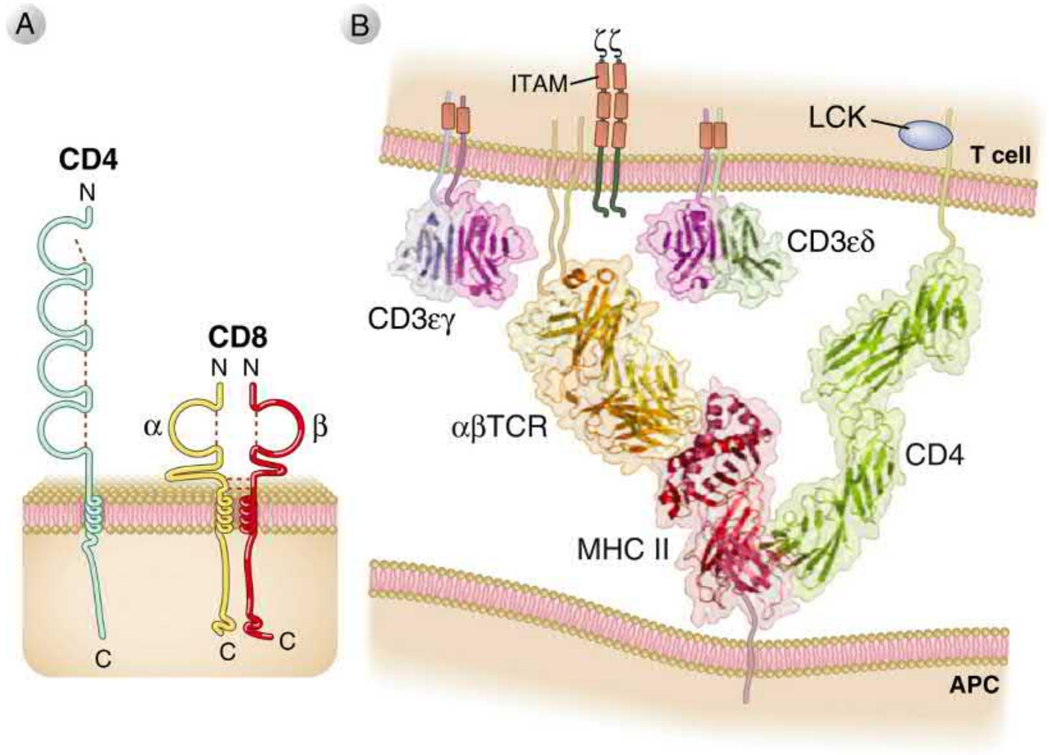


FIGURE 7.10 A Schematic view of the structure of the CD4 and CD8 coreceptors. **A,** The CD4 protein is an integral membrane monomer consisting of four extracellular immunoglobulin (Ig) domains, a transmembrane domain, and a cytoplasmic tail. The CD8 protein is either a disulfide-linked αβ integral membrane heterodimer or a disulfide-linked αα homodimer *(not shown)*. Each chain has a single extracellular Ig domain. **B,** CD4 on T cells associates with an invariant portion of the major histocompatibility complex *(MHC*) class II heterodimer on an antigen-presenting cell *(APC)* that is interacting with the T cell receptor *(TCR)* on the same T cell. Note that the cytoplasmic portions of both CD4 and CD8 can associate with LCK and that the ζ chain is depicted schematically*. ITAM,* Immunoreceptor tyrosine-based activation motif.

Modified from Garcia KC, Adams E. How the T cell receptor sees antigen: a structural view. *Cell.* 2005;122:333–336, 2005; with permission.

Activation of Tyrosine Kinases and a Lipid Kinase During T Cell Activation

Phosphorylation of proteins and lipids plays a central role in the transduction of signals from the TCR complex and coreceptors. Within seconds of TCR ligation, LCK phosphorylates the ITAMs of the CD3 and ζ chains (Fig. 7.11). The tyrosine-phosphorylated ITAMs in the ζ chain are docking sites for the SYK family tyrosine kinase called **ZAP70** (ζ-associated protein of 70 kD). ZAP70 contains two SH2 domains that can bind to ITAM phosphotyrosines. As discussed earlier, each ITAM has two tyrosine residues, and both of these must be phosphorylated to provide a docking site for one ZAP70 molecule. The bound ZAP70 becomes a substrate for the adjacent LCK after TCR recognition of antigen, and LCK phosphorylates specific tyrosine residues of ZAP70. As a result, ZAP70 acquires its own tyrosine kinase activity and is then able to phosphorylate a number of other cytoplasmic signaling molecules. A critical threshold of ZAP70 activity may be needed before downstream signaling events will proceed, and this threshold is achieved by the recruitment of multiple ZAP70 molecules to the phosphorylated ITAMs on the ζ chains and on CD3 tails.

Another signaling pathway in T cells involves the activation of **PI3-kinase** (Fig. 7.12). This enzyme is recruited to adaptor proteins associated with the TCR complex and phosphorylates phosphatidylinositol bisphosphate (PIP2), located in the inner leaflet of the plasma membrane, to generate PIP3. Certain signaling proteins in the cytosol have specialized PH domains that have an affinity for PIP3, and as a result, PH domain–containing proteins can bind to the inside of the cell membrane only when PIP3 is generated. Examples of PH domain–containing proteins include

TEC-family tyrosine kinases such as ITK in T cells and BTK in B cells as well as phospholipase Cγ1 (PLCγ1), a key enzyme in the calcium signaling pathway in T cells. Another important PIP3-dependent kinase is PDK1, a serine/threonine kinase that is required for the phosphorylation and activation of an important downstream kinase called AKT. Activated AKT phosphorylates crucial targets and contributes to both increased protein synthesis and metabolism, leading to cell growth (enlargement) and survival. AKT activates another serine/threonine kinase, called mechanistic target of rapamycin (mTOR), that is a controller of protein synthesis and cell growth. AKT also promotes cell survival in a number of ways, including inactivation of pro-apoptotic proteins and increased production and activation of anti-apoptotic proteins of the BCL-2 family.

Recruitment and Modification of Adaptor Proteins

Activated ZAP70 phosphorylates several adaptor proteins, making them capable of binding to signaling molecules (see Fig. 7.11) **.** A key early event in T cell activation is the ZAP70-mediated tyrosine phosphorylation of adaptor proteins such as SLP76 and LAT. Phosphorylated LAT directly binds PLCγ1 (discussed later) and coordinates the recruitment of several other adaptor proteins, including SLP76, GADS, and GRB2, to the cluster of TCR and TCR-associated proteins, sometimes referred to as the TCR signalosome. Thus, LAT serves to bring downstream components of TCR signaling pathways close to their upstream activators. Because the function of many of these adaptors depends on their tyrosine phosphorylation by active ZAP70, only antigen recognition (the physiologic stimulus for ZAP70 activation) triggers the signal transduction pathways that lead to functional T cell responses.

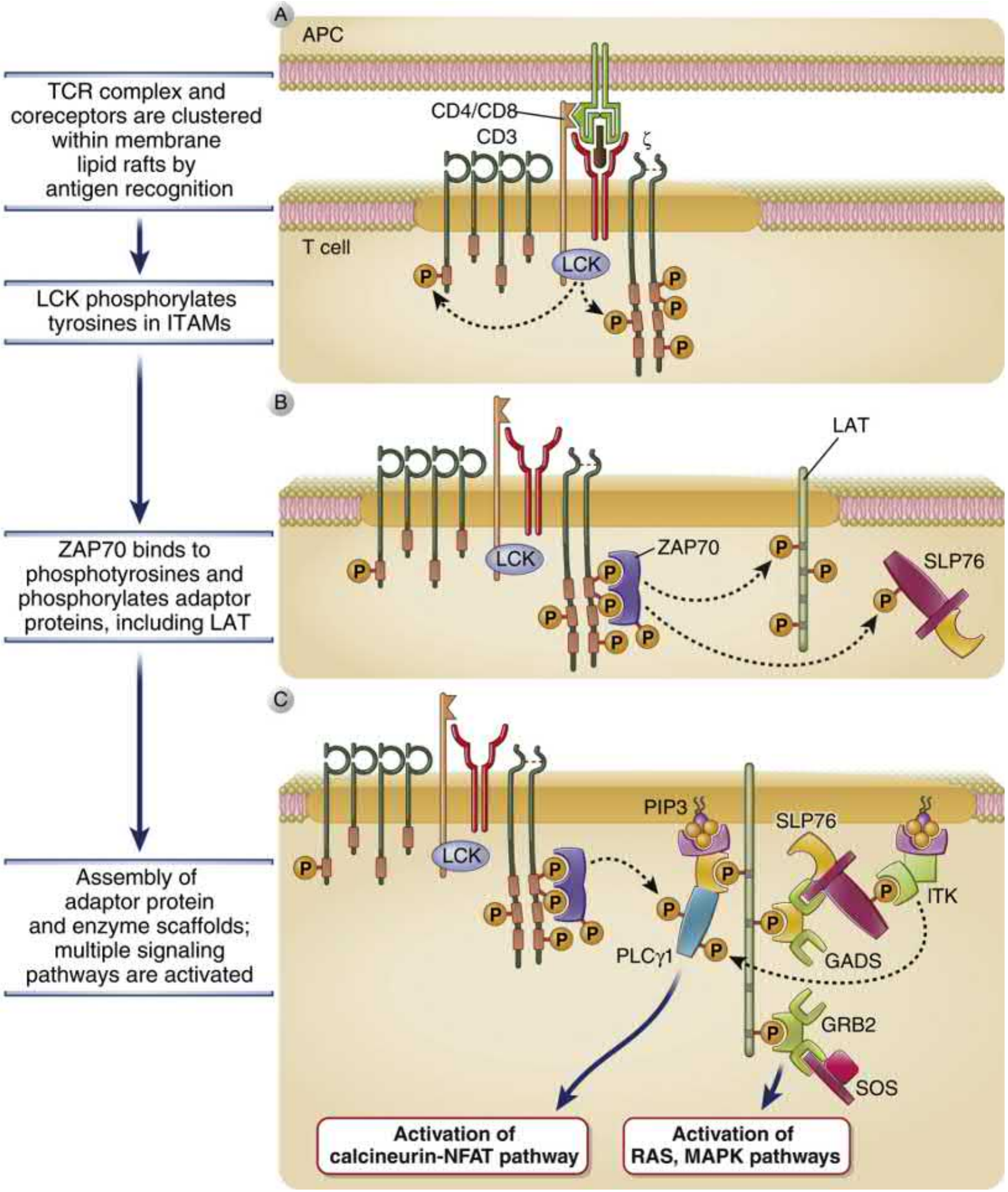

TCR complex and coreceptors are clustered within membrane lipid rafts by antigen recognition
LCK phosphorylates tyrosines in ITAMs
ZAP70 binds to phosphotyrosines and phosphorylates adaptor proteins, including LAT
Assembly of adaptor protein and enzyme scaffolds; multiple signaling pathways are activated
A
APC
CD4/CD8
CD3
ζ
T cell
LCK
P
B
LAT
LCK
ZAP70
SLP76
P
C
PIP3
SLP76
LCK
ITK
PLCγ1
GADS
GRB2
SOS
P
Activation of calcineurin-NFAT pathway
Activation of RAS, MAPK pathways

FIGURE 7.11 Early tyrosine phosphorylation events in T cell activation.On antigen recognition, there is clustering of T cell receptor (TCR) complexes with coreceptors (CD4, in this case). CD4-associated LCK becomes active and phosphorylates tyrosines in the ITAMs of CD3 and ζ chains **(A).** ZAP70 binds to the phosphotyrosines of the ζ chains and is itself phosphorylated and activated. (The illustration shows one ZAP70 molecule

binding to two phosphotyrosines of one ITAM in the ζ chain, but it is likely that initiation of a T cell response requires the assembly of multiple ZAP70 molecules on ITAMs of the two ζ chain as well as on CD3 chains.) Active ZAP70 then phosphorylates tyrosines on various adaptor molecules, such as LAT **(B).** The adaptors become docking sites for cellular enzymes such as PLCγ1 and GDP-GTP exchange factors that activate RAS and other small G proteins upstream of MAPKs **(C),** and these enzymes activate various cellular responses. *GDP,* Guanosine diphosphate; *GTP,* guanosine triphosphate; *ITAM,* immunoreceptor tyrosine-based activation motif; *PLCγ1,* phospholipase C γ1; *MAPK*, mitogen-activated protein kinase.

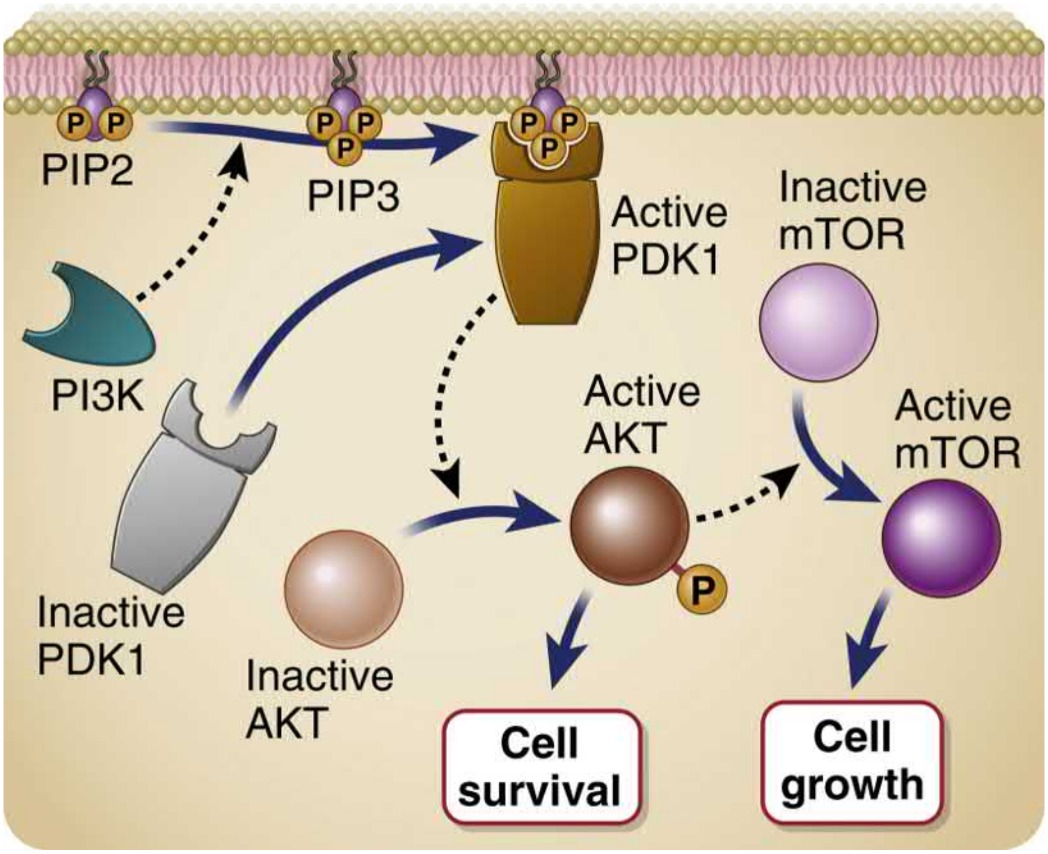

P
PIP2
PIP3
Active
PDK1
Inactive
mTOR
PI3K
Active
AKT
Active
mTOR
Inactive
PDK1
Inactive
AKT
Cell
survival
Cell
growth

FIGURE 7.12 Role of PI3-kinase in T cell responses.Membrane PIP3, generated by PI3K, activates PDK1, which phosphorylates and activates the AKT kinase, which in turn phosphorylates downstream targets that are involved in cell survival and cell growth. *mTOR,* Mechanistic target of rapamycin; *PDK1,* 3-phosphoinositide-dependent kinase 1; *PI3K,* phosphatidylinositol-4,5-bisphosphate 3-kinase; *PIP2,* phosphatidylinositol bisphosphate; *PIP3,* phosphatidylinositol trisphosphate.

Formation of the Immune Synapse

When the TCR complex recognizes MHC-associated peptides on an APC, several T cell surface proteins and intracellular signaling molecules are rapidly mobilized to the site of T cell–APC contact

(Fig. 7.13) . This region of physical contact between the T cell and the APC forms a bull's eye–like structure that is called an **immune synapse** or a supramolecular activation cluster (SMAC). The T cell molecules that move to the center of the synapse include the TCR complex (the TCR, CD3, and ζ chains), CD4 or CD8 coreceptors, receptors for costimulators (such as CD28), enzymes such as PKCθ, and adaptor proteins that associate with the cytoplasmic tails of the transmembrane receptors. At this region of the synapse, called the c-SMAC (for central SMAC), the distance between plasma membranes of the T cell and the APC is about 15 nm. Integrins remain at the periphery of the synapse, where they function to stabilize the binding of the T cell to the APC, forming the peripheral portion of the SMAC, called the p-SMAC. In this outer part of the synapse, the two membranes are about 40 nm apart. Many signaling molecules found in synapses are initially localized to regions of the plasma membrane that have a lipid content different from that of the rest of the cell membrane and are called lipid rafts or glycolipid-enriched microdomains. TCR and costimulatory receptor signaling is initiated in these rafts, and signaling initiates cytoskeletal rearrangements that allow rafts to coalesce and form the immune synapse.

Immune synapses serve a number of functions during and after T cell activation.

- The synapse forms a stable contact between an antigen-specific T cell and an APC displaying that antigen and becomes the site for assembly of the signaling machinery of the T cell, including the TCR complex, coreceptors, costimulatory receptors, and adaptors. Although some TCR signal transduction is initiated before the formation of the synapse and is, in fact, required for synapse formation, the immune synapse itself provides a unique interface for TCR triggering. T cell activation needs to overcome the problems of a generally low affinity of TCRs for peptide-MHC ligands and the presence of few MHC molecules displaying any one peptide on an APC. The synapse represents a site at which repeated engagement of TCRs can be sustained by this small number of peptide-MHC complexes on the APC, thus facilitating prolonged and effective T cell signaling.

- The synapse ensures the specific delivery of secretory granule contents and cytokines from a T cell to APCs or to targets that are in contact with the T cell. Vectorial delivery of secretory granules containing perforin and granzymes from CTLs to target cells occurs at the synapse (see Chapter 11). Similarly, CD40L-CD40 interactions are facilitated by the accumulation of these molecules on the T cell and APC interfaces of the immune synapse. Some cytokines are also secreted in a directed manner into the synaptic cleft, from where they are preferentially delivered to the cell that is displaying antigen to the T lymphocyte. This ensures that cytokines act on cells bearing foreign (e.g., microbial) antigens, which are the cells that need to be activated or killed to eliminate these antigens.
- The synapse, especially the c-SMAC region, also may be an important site for the turnover of signaling molecules, primarily by ubiquitination and delivery to late endosomes and lysosomes. This degradation of signaling proteins contributes to the termination of T cell activation and is discussed later.

MAP Kinase Signaling Pathways in T Lymphocytes

Small guanine nucleotide–binding proteins (G proteins) activated by antigen recognition stimulate at least three different mitogen-activated protein (MAP) kinases, which in turn activate distinct transcription factors. G proteins are involved in diverse activation responses in different cell types. Two major members of this family activated downstream of the TCR are RAS and RAC. Each activates a different transcription factor, and together they mediate many cellular responses of T cells.

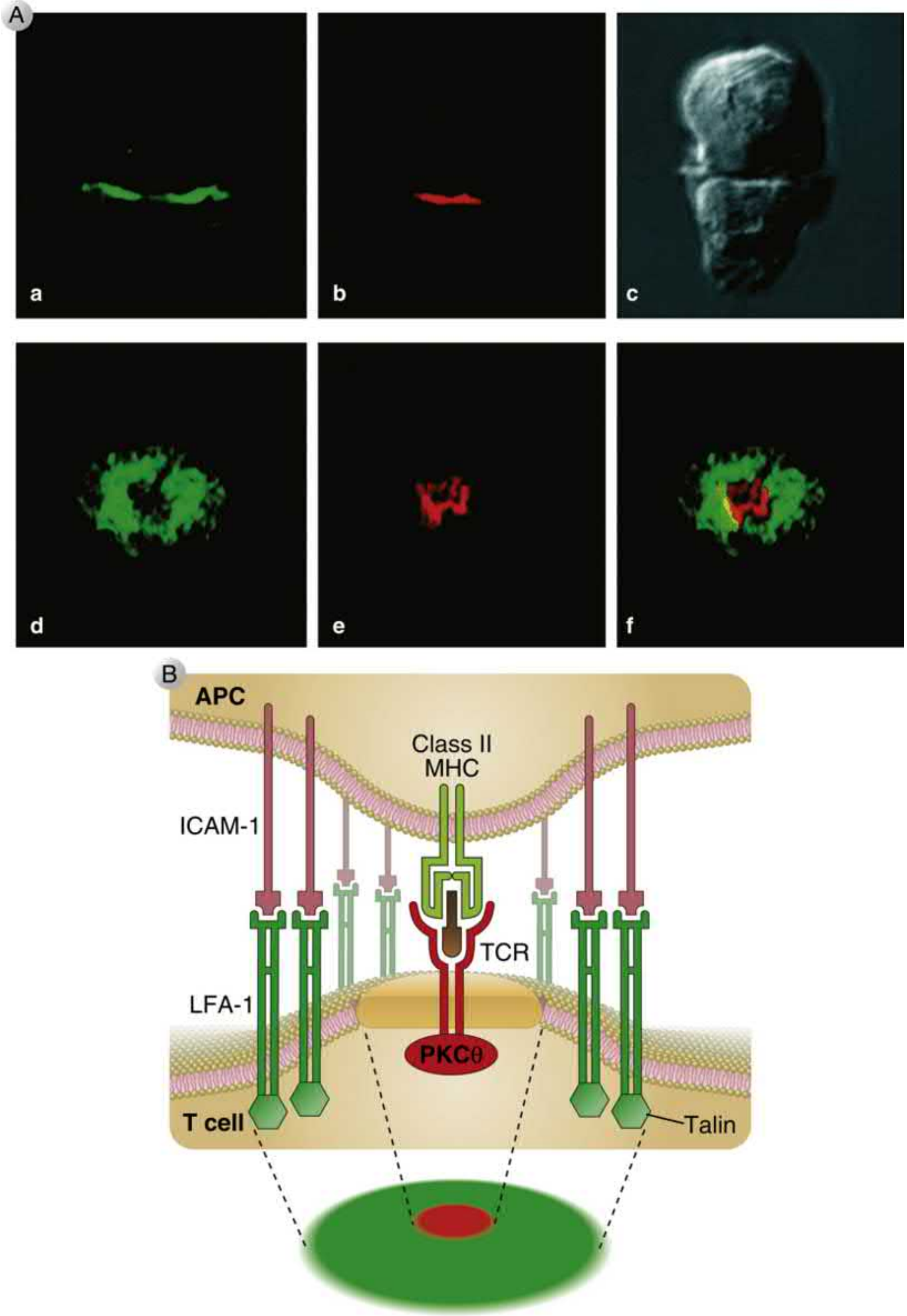

A
a
b
c
d
e
f
B
APC
Class II MHC
ICAM-1
TCR
LFA-1
PKCθ
T cell
Talin

FIGURE 7.13 The immune synapse. **A,** This figure shows two views of the immunologic synapse in a T cell–APC conjugate (shown as a Nomarski

image in *panel c*). Talin, a protein that associates with the cytoplasmic tail of the LFA-1 integrin, was revealed by an antibody labeled with a green fluorescent dye, and PKCθ, which associates with the TCR complex, was visualized by antibodies conjugated to a red fluorescent dye. In *panels a and b,* a two-dimensional optical section of the cell contact site along the *x-y* axis is shown, revealing the central location of PKCθ and the peripheral location of talin, both in the T cell. In *panels d to f,* a three-dimensional view of the entire region of cell-cell contact along the *x–z* axis is provided. Note, again, the central location of PKCθ and the peripheral accumulation of talin. **B,** A schematic view of the synapse, showing talin and LFA-1 in the p-SMAC *(green)* and PKCθ and the TCR in the c-SMAC *(red)*. *APC,* Antigen-presenting cell; *ICAM-1,* intercellular adhesion molecule 1; *MHC,* major histocompatibility complex; *TCR,* T cell receptor.

A, Reprinted with permission of Macmillan Publishers Ltd. from Monks CRF, Freiburg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature.* 1998;395:82–86. Copyright 1998.

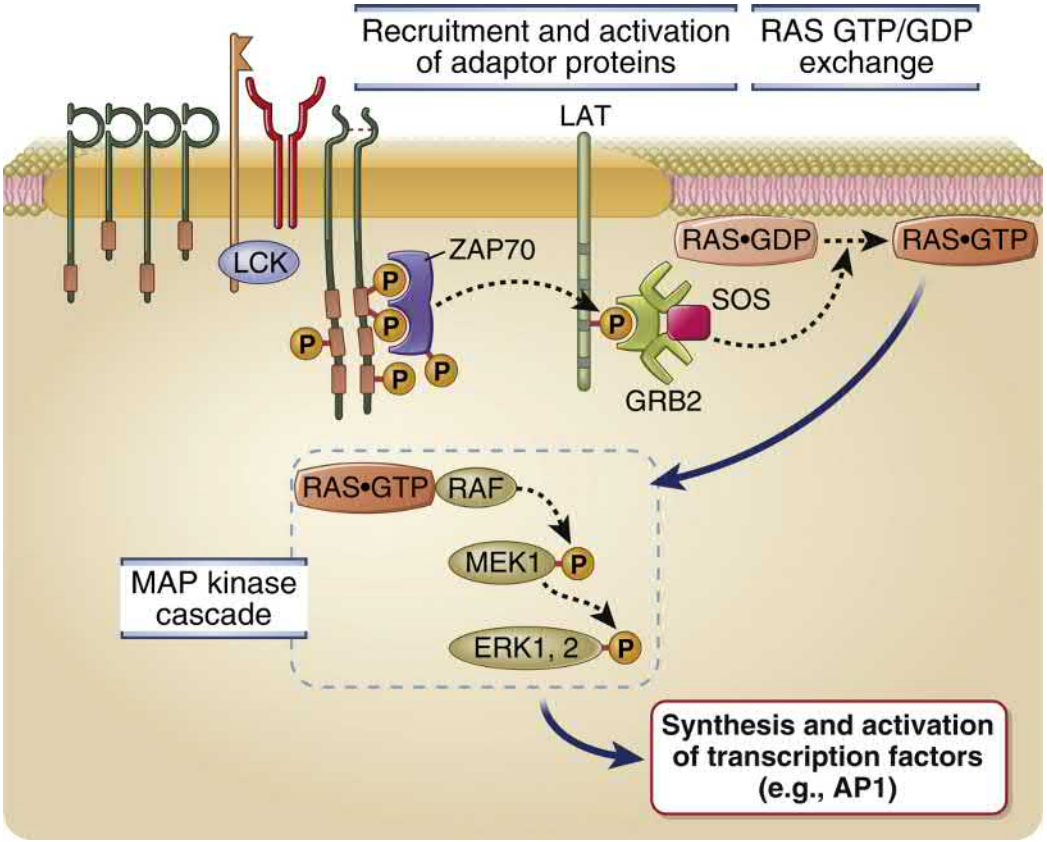

Recruitment and activation of adaptor proteins
RAS GTP/GDP exchange
LAT
LCK
ZAP70
P
RAS•GDP
RAS•GTP
SOS
GRB2
RAS•GTP
RAF
MEK1
ERK1, 2
MAP kinase cascade
Synthesis and activation of transcription factors (e.g., AP1)

FIGURE 7.14 The RAS-MAP kinase pathway in T cell activation.ZAP70, activated by antigen recognition, phosphorylates membrane-associated adaptor proteins (such as LAT) that then bind another adaptor, GRB2, which provides a docking site for the GTP-GDP exchange factor SOS. SOS converts RAS·GDP to RAS·GTP. RAS·GTP activates a cascade of enzymes, which culminates in the activation of the MAP kinase ERK. A parallel RAC-dependent pathway generates another active MAP kinase, JNK *(not shown)*. *AP1,* Activator protein 1; *GDP,* guanosine diphosphate; *GTP,* guanosine triphosphate; *MAP,* mitogen-activated protein.

- The **RAS** pathway is triggered in T cells after TCR ligation, leading to the activation of extracellular receptor–activated

> kinase (ERK), a prominent member of the MAP kinase family, and eventually to the activation of downstream transcription factors (Fig. 7.14). RAS is loosely attached to the plasma membrane through covalently bound lipids. In its inactive form, the guanine nucleotide–binding site of RAS is occupied by guanosine diphosphate (GDP). Upon activation of the cell, the bound GDP is replaced by guanosine triphosphate (GTP), and RAS undergoes a conformational change and can then recruit or activate various cellular enzymes, the most important of which is RAF. Activation of RAS by GDP/GTP exchange is seen in response to the engagement of many types of receptors in many cell populations, including the TCR complex in T cells. Nonmutated RAS proteins are active GTPases that convert the GTP bound to RAS into GDP, thus returning RAS to its normal, inactive state. Mutated RAS proteins that are constitutively active (i.e., they constantly assume the GTP-bound conformation, often because of loss of GTPase activity) contribute to the neoplastic transformation of many cell types.

The mechanism of RAS activation in T cells involves the adaptor proteins LAT and GRB2. When LAT is phosphorylated by ZAP70 at the site of TCR clustering, it serves as the docking site for the SH2 domain of GRB2. Once attached to LAT, GRB2 recruits the RAS GTP/GDP exchange factor called SOS to the plasma membrane. SOS catalyzes GTP for GDP exchange on RAS. This generates the GTP-bound form of RAS (written as RAS·GTP), which then triggers a cascade of kinase activation. RAS·GTP directly activates a kinase called RAF, the first kinase in this cascade. RAF then phosphorylates and activates a dual-specificity kinase called MEK1, which in turn phosphorylates the third kinase in the cascade, called ERK, on closely spaced threonine and tyrosine residues. ERK is a MAP kinase, and MEK1 is called a MAP kinase-kinase (a kinase that activates a MAP kinase). The activated ERK translocates to the nucleus and phosphorylates a protein called ELK, and phosphorylated ELK stimulates transcription of FOS, a component of the activation protein 1 (AP1) transcription factor.

- In parallel with the activation of RAS through recruitment of GRB2 and SOS, the adaptors phosphorylated by TCR-associated kinases also recruit and activate a GTP/GDP exchange protein called **VAV** that acts on **RAC**, another small G protein. The RAC·GTP that is generated initiates a parallel MAP kinase cascade, resulting in the activation of a distinct MAP kinase, c-JUN N-terminal kinase (JNK). JNK is sometimes called a stress-activated protein (SAP) kinase because in many cells it is activated by various noxious stimuli. Activated JNK then phosphorylates JUN, the second component of the AP1 transcription factor. A third member of the MAP kinase family, in addition to ERK and JNK, is p38, and it too is activated by RAC·GTP and in turn activates various transcription factors. RAC·GTP also induces cytoskeletal reorganization and may play a role in the clustering of TCR complexes, coreceptors, and other signaling molecules into the synapse.

The activities of ERK and JNK are eventually shut off by the action of dual-specificity protein tyrosine/threonine phosphatases. These phosphatases are induced or activated by ERK and JNK themselves, providing a negative feedback mechanism to terminate T cell activation.

Calcium- and Protein Kinase C–Mediated Signaling Pathways in T Lymphocytes

TCR signaling leads to the activation of the γ1 isoform of the enzyme phospholipase C (PLCγ1), and the products of PLCγ1-mediated hydrolysis of membrane lipids activate additional signaling events that induce specific transcription factors in T cells (Fig. 7.15)**.** Soon after TCR triggering, the LAT and SLP76 adaptors are phosphorylated on tyrosine residues by ZAP70 and form a complex on the inside of the plasma membrane. At the same time, PI3-kinase generates PIP3 in the inner leaflet of the plasma membrane and recruits the TEC-family kinase, ITK, and also PLCγ1 (both enzymes have PH domains and can thus bind to PIP3). These enzymes also contain SH2 domains that allow them to bind to specific phosphorylated tyrosine residues on the LAT/SLP76 complex, and it is here that ITK phosphorylates and activates PLCγ1. Activated PLCγ1 catalyzes the hydrolysis of the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2), generating two breakdown products: the soluble sugar triphosphate, inositol 1,4,5-trisphosphate (IP3), and membrane-bound diacylglycerol (DAG). IP3 and DAG then activate two distinct downstream signaling pathways in T cells.

IP3 produces a rapid increase in cytosolic free calcium after T cell activation. IP3 diffuses through the cytosol to the smooth endoplasmic reticulum (ER), where it binds to its receptor, a ligand-gated calcium channel, and stimulates release of membrane-sequestered calcium stores. As a result, the cytosolic free calcium ion concentration increases from a resting level of about 100 nM to a peak of 600 to 1000 nM within a few minutes. The depletion of ER calcium is sensed by STIM1, an ER membrane protein, which then activates a plasma membrane ion channel called a calcium release–activated calcium (CRAC) channel. Portions of the ER membrane in the cell are often very close to the plasma membrane and this proximity facilitates the interaction between STIM1 and the CRAC channel. The activation of the CRAC channel results in an influx of extracellular calcium that sustains cytosolic levels at about 300 to 400 nM for more than 1 hour. A key component of the CRAC channel is the ORAI protein; mutations in the gene encoding this protein are the cause of a rare human immunodeficiency disease. Cytosolic free calcium acts as a signaling molecule by binding to calmodulin, a ubiquitous calcium-dependent regulatory protein. Calcium-calmodulin complexes activate several enzymes, including calcineurin, a protein serine/threonine phosphatase that is important for transcription factor activation, as discussed later.

DAG, the second breakdown product of PIP2, is a membrane-bound lipid that activates the enzyme protein kinase C (PKC). There are several isoforms of PKC that participate in the generation of active transcription factors, discussed later. The combination of elevated free cytosolic calcium and DAG induces a conformational change in certain isoforms of PKC that makes the catalytic site accessible to its substrates. Numerous downstream proteins are phosphorylated by PKC. The PKCθ isoform localizes to the immune synapse and is involved in the activation and nuclear translocation of the nuclear factor κB (NF-κB) transcription factor. Pathways of NF-κB activation are discussed later in this chapter.

So far, we have described several signal transduction pathways initiated by ligand binding to the TCR that result in the activation of different types of enzymes: G protein–MAP kinase pathways leading to activation of kinases such as ERK and JNK, a PLCγ1-calcium–dependent pathway leading to activation of the phosphatase calcineurin, and a DAG-dependent pathway leading to activation of PKC. Each of these pathways contributes to the expression of genes encoding proteins needed for T cell clonal expansion, differentiation, and effector functions. In the following section, we will describe the mechanisms by which these different signaling pathways stimulate the transcription of various genes in T cells.

Activation of Transcription Factors That Regulate T Cell Gene Expression

The enzymes generated by TCR signaling activate transcription factors that bind to regulatory regions of numerous genes in T cells and thereby enhance transcription of these genes (Fig. 7.16) **.** Much of our understanding of the transcriptional regulation of genes in T cells is based on analyses of cytokine gene expression. The transcriptional regulation of most cytokine genes in T cells is controlled by the binding of transcription factors to nucleotide sequences in the promoter and enhancer regions of these genes.

For instance, the promoter located 5′ of the coding exons of the interleukin-2 *(IL2)* gene contains a segment of approximately 300 base pairs that contains binding sites for several different transcription factors. All of these sites must be occupied by transcription factors for maximal expression of the *IL2* gene. Different transcription factors are activated by different cytoplasmic signal transduction pathways, and the requirement for multiple transcription factors accounts for the need to activate many signaling pathways after antigen recognition. The same principles are true for the induced expression of many other genes in T cells, including those encoding cytokine receptors and effector molecules, although different genes may be responsive to different combinations of transcription factors.

Three transcription factors that are activated in T cells by antigen recognition and appear to be critical for most T cell responses are nuclear factor of activated T cells (NFAT), AP1, and NF-κB.

- **NFAT** is a transcription factor required for the expression of genes encoding IL-2, IL-4, TNF, and other cytokines. NFAT is present in an inactive, serine-phosphorylated form in the cytoplasm of resting T lymphocytes. It is activated by the calcium-calmodulin–dependent phosphatase **calcineurin.** Calcineurin dephosphorylates cytoplasmic NFAT, thereby uncovering a nuclear localization signal that permits NFAT to translocate into the nucleus. Once it is in the nucleus, NFAT binds to the regulatory regions of the *IL2* and other genes, usually in association with other transcription factors, such as AP1. The mechanism of activation of NFAT was discovered indirectly by studies of the mechanism of action of the immunosuppressive drug cyclosporine. This drug and the functionally similar compound tacrolimus (FK506) are natural products of fungi that are used as therapeutic agents to treat transplant rejection (see Chapter 17). They function largely by blocking T cell cytokine gene transcription. Cyclosporine binds to a cytosolic protein called cyclophilin, and tacrolimus binds to a protein called

FK506-binding protein (FKBP). Cyclosporine-cyclophilin complexes and tacrolimus-FKBP complexes bind to and inhibit calcineurin (hence these drugs are called calcineurin inhibitors) and thereby block translocation of NFAT into the nucleus.

- **AP1** is a transcription factor found in many cell types; it is specifically activated in T lymphocytes by TCR-mediated signals. AP1 is actually the name for a family of DNA-binding factors composed of dimers of two proteins that bind to one another through a shared structural motif called a leucine zipper. The best characterized AP1 factor is composed of the proteins FOS and JUN. As discussed previously, the formation of active AP1 typically involves synthesis of the FOS protein and phosphorylation of preexisting JUN protein, both stimulated by MAP kinases that are activated by TCR-induced signals. AP1 physically associates with other transcription factors in the nucleus, and it works best in combination with NFAT. Thus, AP1 activation represents a convergence point of several TCR-initiated signaling pathways.
- **NF-κB** refers to a group of closely related transcription factors that are activated in response to TCR signals and are essential for cytokine synthesis. NF-κB proteins are homodimers or heterodimers of proteins that are homologous to c-REL and are important in the transcription of many genes in diverse cell types. The NF-κB pathway is important not only for antigen receptor–mediated lymphocyte activation but also for responses to signaling by innate pattern recognition receptors such as Toll-like receptors (TLRs) and cytokine receptors and is discussed in depth at the end of this chapter.

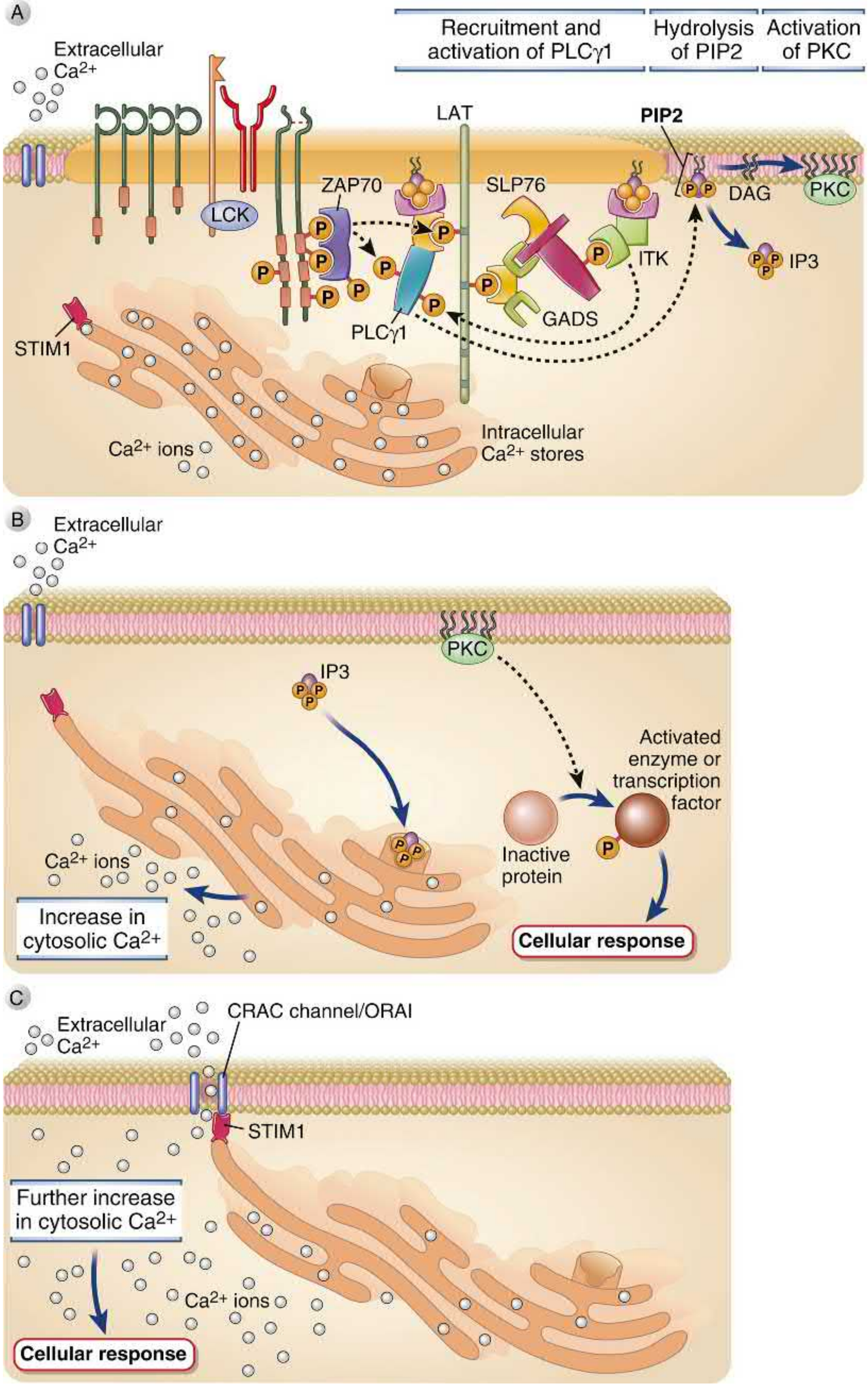

A
Recruitment and activation of PLCγ1
Hydrolysis of PIP2
Activation of PKC
Extracellular Ca2+
LAT
PIP2
ZAP70
SLP76
DAG
PKC
LCK
P
ITK
IP3
GADS
PLCγ1
STIM1
Intracellular Ca2+ stores
Ca2+ ions
B
Extracellular Ca2+
PKC
IP3
Activated enzyme or transcription factor
Inactive protein
Ca2+ ions
Increase in cytosolic Ca2+
Cellular response
C
Extracellular Ca2+
CRAC channel/ORAI
STIM1
Further increase in cytosolic Ca2+
Ca2+ ions
Cellular response

FIGURE 7.15 T cell signaling downstream of PLCγ1. **A,** The LAT adaptor protein that is phosphorylated on T cell activation binds the cytosolic enzyme PLCγ1, which is phosphorylated by ITK and activated. Active PLCγ1 hydrolyzes membrane PIP2 to generate IP3, which stimulates an increase in cytosolic calcium, and DAG, which activates the enzyme PKC. **B,** IP3 causes depletion of endoplasmic reticulum calcium, which is sensed by STIM1. PKC induces numerous cellular responses. **C,** STIM1 induces the opening of the CRAC channel that facilitates entry of extracellular calcium into the cytosol. ORAI is a component of the CRAC channel. Increased cytosolic calcium together with PKC activate various transcription factors, leading to cellular responses. *CRAC,* Calcium release–activated calcium; *DAG,* diacylglycerol; *IP3,* inositol 1,4,5-trisphosphate; *PIP2,* phosphatidylinositol bisphosphate; *PKC,* protein kinase C; *PLCγ1,* phospholipase C γ1.

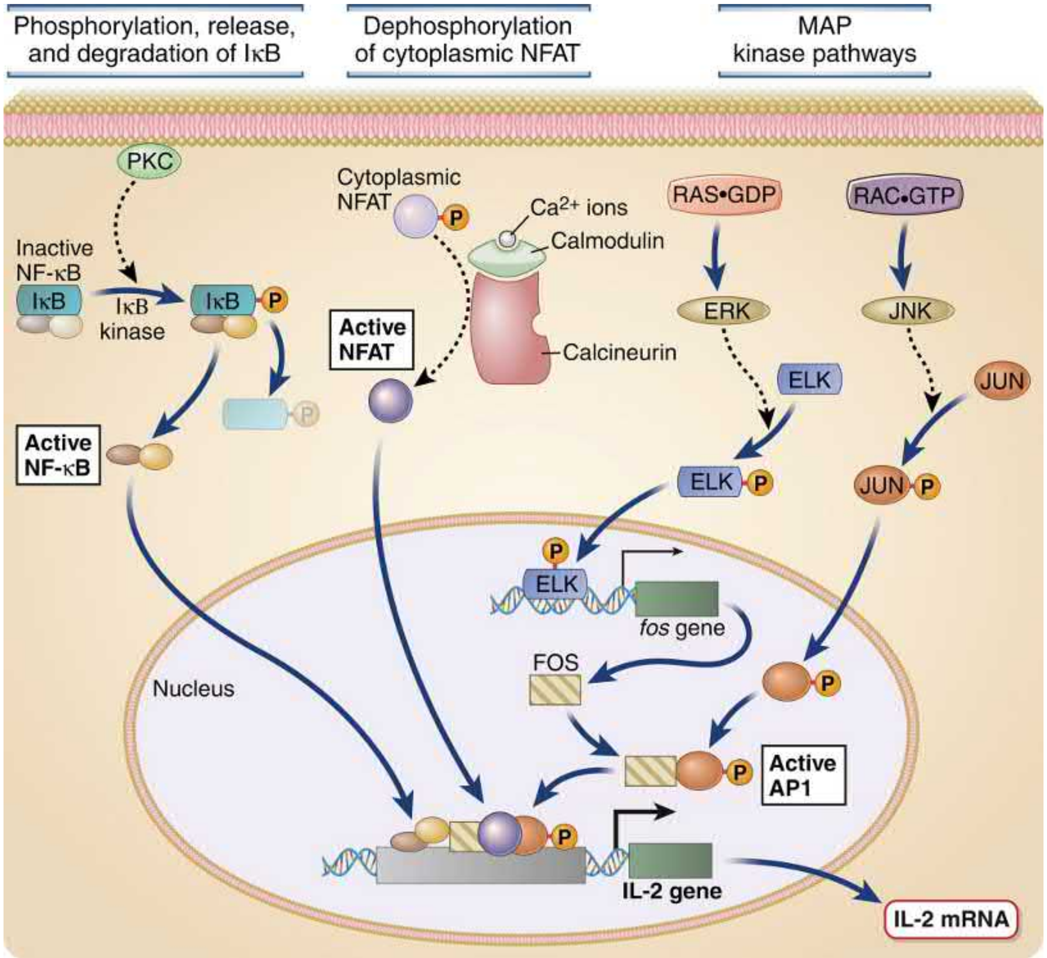

Phosphorylation, release, and degradation of IκB
Dephosphorylation of cytoplasmic NFAT
MAP kinase pathways
PKC
Inactive NF-κB
IκB
IκB kinase
IκB
P
P
Active NF-κB
Cytoplasmic NFAT
P
Ca2+ ions
Calmodulin
Active NFAT
Calcineurin
RAS•GDP
RAC•GTP
ERK
JNK
ELK
JUN
ELK
P
JUN
P
P
ELK
fos gene
FOS
P
Nucleus
P
Active AP1
P
IL-2 gene
IL-2 mRNA

FIGURE 7.16 Activation of transcription factors in T cells.Multiple signaling pathways converge in antigen-stimulated T cells to generate transcription factors that stimulate expression of various genes (in this case, the *IL2* gene). The calcium-calmodulin pathway activates NFAT, and the RAS and RAC pathways generate the two components of AP1. Less is known about the link between TCR signals and NF-κB activation. (NF-κB is shown as a complex of two subunits, which in T cells are typically the p50 and p65 proteins, named for their molecular sizes in kilodaltons.) PKC is important in T cell activation, and the PKCθ isoform is particularly important in activating NF-κB. These transcription factors function

coordinately to regulate gene expression. Note also that the various signaling pathways are shown as activating unique transcription factors, but there may be considerable overlap, and each pathway may play a role in the activation of multiple transcription factors. *AP1,* Activator protein 1; *GDP,* guanosine diphosphate; *GTP,* guanosine triphosphate; *IL-2,* interleukin-2; *NFAT,* nuclear factor of activated T cells; *PKC,* protein kinase C; *TCR,* T cell receptor.

The links between different signaling proteins, activation of transcription factors, and functional responses of T cells are often difficult to establish because there are complex and incompletely understood interactions between signaling pathways. In addition, for the sake of simplicity, we often discuss signaling as a set of linear pathways, but we know this does not reflect the more complex and interconnected reality. Finally, we have focused on selected pathways to illustrate how antigen recognition may lead to biochemical alterations, but it is clear that many other signaling molecules are also involved in antigen-induced lymphocyte activation.

An additional mechanism by which T cell activation is regulated involves **microRNAs (miRNAs)**, which are responsible for posttranscriptional inhibition of gene expression. miRNAs are small noncoding RNAs that are transcribed from DNA but are not translated into proteins. They are initially generated in the nucleus as longer primary transcripts that are processed by an endoribonuclease called Drosha into shorter pre-miRNAs that have a stem loop structure and can be exported into the cytosol. In the cytosol, pre-miRNAs are processed by another endoribonuclease called Dicer into short double-stranded miRNAs, 21 to 22 base pairs in length, which associate with several proteins, including Argonaute, to form complexes known as RNA-induced silencing complexes (RISC). One strand of the miRNA can pair with a complementary sequence in a number of cellular messenger RNAs (mRNAs). If the 6– to 8–base pair miRNA sequence that recognizes mRNAs is perfectly complementary to the mRNA, the mRNA may be targeted for degradation, but if the complementarity is imperfect, translation of the mRNA is inhibited. In either case, the result is a reduction in the abundance of proteins encoded by genes targeted by miRNAs. In T cells the expression of the majority of miRNAs is globally reduced upon activation. In addition, the Argonaute protein is ubiquitinated and degraded, further compromising miRNA function and enhancing the expression of a large number of proteins required for cell cycle progression downstream of T cell activation.

Modulation of T Cell Signaling by Protein Tyrosine Phosphatases

Tyrosine phosphatases remove phosphate moieties from tyrosine residues on proteins and generally inhibit TCR signaling. Two tyrosine phosphatases that serve an important inhibitory role in lymphocytes and other hematopoietic cells are called SHP1 and SHP2 (for SH2 domain–containing phosphatases 1 and 2). Inhibitory phosphatases are typically recruited to ITIMs in the cytoplasmic tails of inhibitory receptors that are themselves phosphorylated by tyrosine kinases induced during lymphocyte activation. These phosphatases inhibit signal transduction by removing phosphate moieties from tyrosine residues in key signaling molecules and thus functionally antagonize tyrosine kinases. Another inhibitory phosphatase called SHIP (SH2 domain–containing inositol phosphatase) does not act on phosphoproteins but rather is specific for an inositol phospholipid. Like SHP1 and SHP2, SHIP binds to phosphorylated ITIM sequences on specific inhibitory receptors. SHIP removes a phosphate group from PIP3 in the inner leaflet of the plasma membrane and thus antagonizes PI3-kinase signaling.

Although most phosphatases attenuate lymphocyte signaling, one tyrosine phosphatase, CD45, facilitates lymphocyte activation.

The CD45 protein is a receptor tyrosine phosphatase expressed in all hematopoietic cells. It is an integral membrane protein whose cytoplasmic tail contains tandem protein tyrosine phosphatase domains. CD45 dephosphorylates inhibitory tyrosine residues in SRC family kinases (including LCK and FYN in T cells) and thus contributes to the generation of active kinases.

Costimulatory Receptor Signaling in T Cells

Costimulatory signals are generated by receptors that recognize ligands on APCs and cooperate with TCR signals to promote activation of the T cells. The two-signal hypothesis for T cell activation was introduced in Chapters 1 and 4. In immunologic jargon, the response by the TCR to MHC and peptide on an APC is referred to as signal 1. T cells are fully activated only when a foreign peptide bound to an MHC molecule is recognized in the context of activation of the innate immune system by a pathogen or some other cause of inflammation. Costimulatory ligands represent the danger signals (or signal 2) induced on APCs by microbes. Thus, recognition of foreign antigens must be combined with a sense of danger for optimal T cell activation to occur.

The CD28 Family of Costimulatory Receptors

The best defined costimulators for T lymphocytes are a pair of related proteins, called B7-1 (CD80) and B7-2 (CD86), which are expressed on activated dendritic cells (DCs) and other APCs and bind to the CD28 receptor on T cells. The **CD28** molecule is the principal costimulatory receptor for delivery of second signals for T cell activation. The biologic roles of the B7 and CD28 protein families are discussed in Chapter 9. Another activating receptor of the CD28 family is a molecule called inducible costimulator (ICOS), which plays an important role in T follicular helper cell development and will be discussed in Chapter 12.

The CD2/SLAM Family of Costimulatory Receptors

Proteins other than CD28 family members also contribute to T cell activation and differentiation. The CD2 family consists of homologous proteins that play a role in the activation of T cells and NK cells. In human T cells, CD2 functions both as an intercellular adhesion molecule and as a signal transducer.

A distinct subgroup of the CD2 family of proteins is known as the **SLAM** (signaling lymphocytic activation molecule) family. SLAM, like all members of the CD2 family, is an integral membrane protein that contains two extracellular Ig domains and a long cytoplasmic tail. The cytoplasmic tail of SLAM, but not of CD2, contains a switch motif (ITSM) that is distinct from the ITAM and ITIM motifs found in other activating and inhibitory receptors.

The extracellular Ig domains of SLAM are involved in homophilic interactions. SLAM on a T cell can interact with SLAM on a DC, and as a result the cytoplasmic tail of SLAM may deliver signals to T cells. The ITSM motif binds to SAP (SLAM-associated protein), and the latter forms a bridge between SLAM and FYN (a SRC family kinase that is also physically linked to CD3 proteins in T cells). SLAM and other members of the SLAM family function as costimulatory receptors in T cells, NK cells, and some B cells. As we will discuss in Chapter 21, mutations in the *SH2D1A* gene encoding SAP are the cause of a disease called **X-linked lymphoproliferative syndrome (XLP).**

An important member of the SLAM family in NK cells, $CD8^+$ T cells, and $\gamma\delta$ T cells is called **2B4.** Like SLAM, the cytoplasmic tail of 2B4 contains ITSMs, binds to the SAP adaptor protein, and induces activating signals by recruiting FYN. Defective 2B4 signaling contributes to the immune deficit in patients with X-linked lymphoproliferative syndrome (see Chapter 21).

Metabolic Changes During T Cell Activation

When cells are activated, they need to increase their metabolic activity to cope with the increased demands of the cellular response. In the immune system this phenomenon has been best studied in T cells. Upon activation by antigen and costimulators, T cells increase the transport of glucose and change their energy production from mitochondrial oxidative phosphorylation to glycolysis, even in the presence of abundant oxygen, a phenomenon known as aerobic glycolysis, or the Warburg effect (Fig. 7.17). This was first described in tumor cells but is now recognized as an important mechanism used by many proliferating cells. Although glycolysis generates less ATP, the molecule cells use to store and release energy, than oxidative phosphorylation, glycolysis does not use substrates other than glucose, such as amino acids and lipids, and thus preserves essential building blocks needed for the synthesis of new macromolecules and for cell division. Aerobic glycolysis in lymphocytes may be important not just for cellular proliferation but also for the differentiation of T cells into effector cells and for the production of effector cytokines.

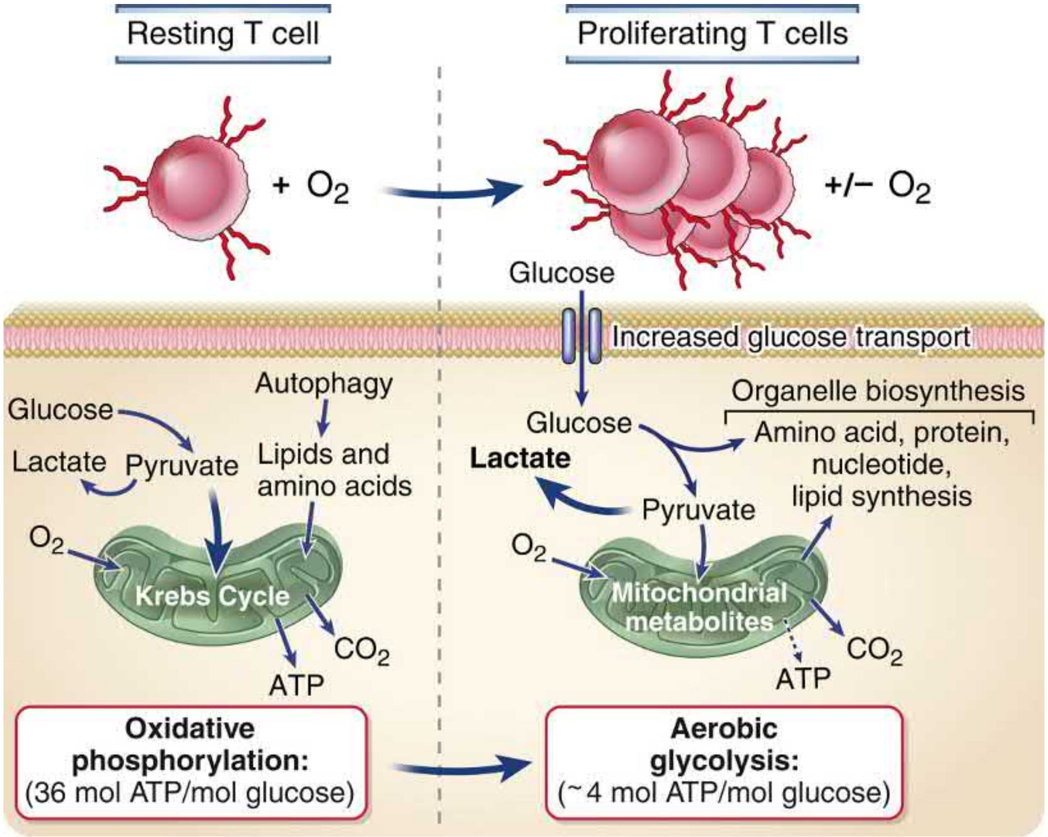

Resting T cell
Proliferating T cells
+ O_2
+/− O_2
Glucose
Increased glucose transport
Autophagy
Glucose
Lactate
Pyruvate
Lipids and amino acids
O_2
Krebs Cycle
CO_2
ATP
Organelle biosynthesis
Glucose
Amino acid, protein, nucleotide, lipid synthesis
Lactate
Pyruvate
O_2
Mitochondrial metabolites
CO_2
ATP
Oxidative phosphorylation:
(36 mol ATP/mol glucose)
Aerobic glycolysis:
(~4 mol ATP/mol glucose)

FIGURE 7.17 Metabolic changes during T cell activation. In resting T cells, the major pathway of energy generation is mitochondrial oxidative phosphorylation. Upon activation, there is a switch to aerobic glycolysis, which generates less energy but preserves and produces the building blocks for cellular organelle biosynthesis, which is required for cell proliferation and functional responses. *ATP,* Adenosine triphosphate.

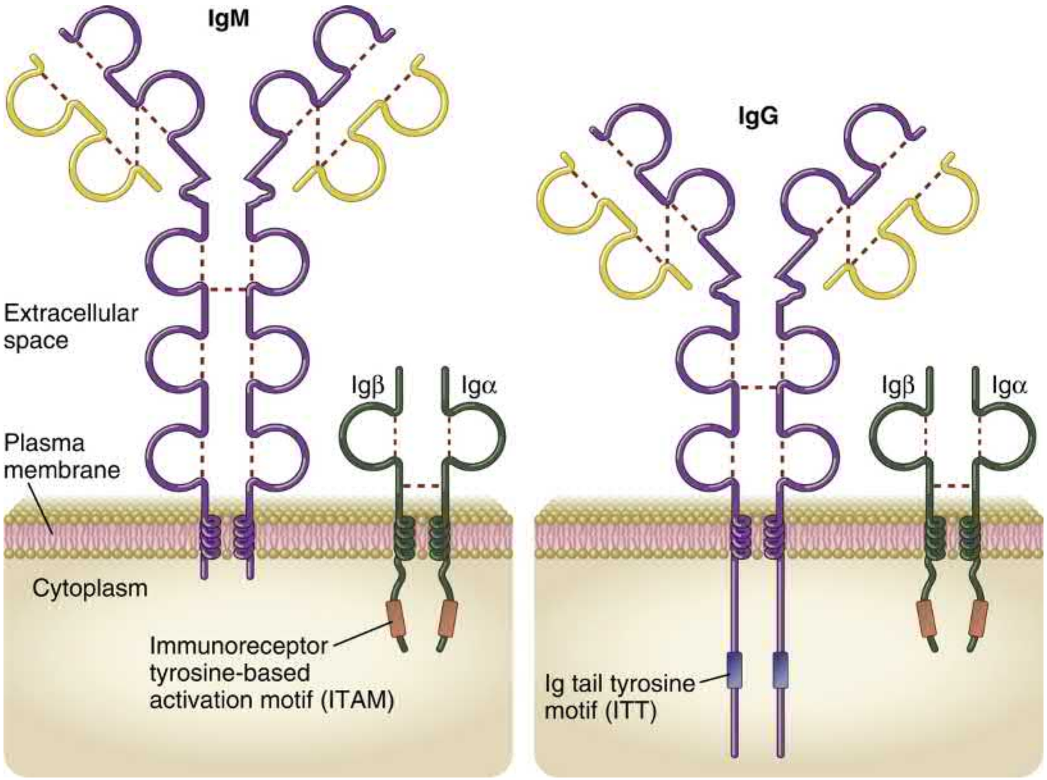

IgM
IgG
Extracellular space
Plasma membrane
Cytoplasm
Igβ
Igα
Igβ
Igα
Immunoreceptor tyrosine-based activation motif (ITAM)
Ig tail tyrosine motif (ITT)

FIGURE 7.18 B cell antigen receptor complex.As seen in the *left panel*, membrane immunoglobulin *(IgM)* (and *IgD*) on the surface of mature B cells is associated with the invariant *Igβ* and *Igα* molecules, which contain ITAMs in their cytoplasmic tails that mediate signaling functions. In the *right panel* the cytoplasmic tail of membrane IgG (and the same is true for membrane IgE) contains a tyrosine containing motif called the Ig tail tyrosine (ITT) motif that helps amplify B cell receptor signaling in memory B cells.

The B Lymphocyte Antigen Receptor Complex

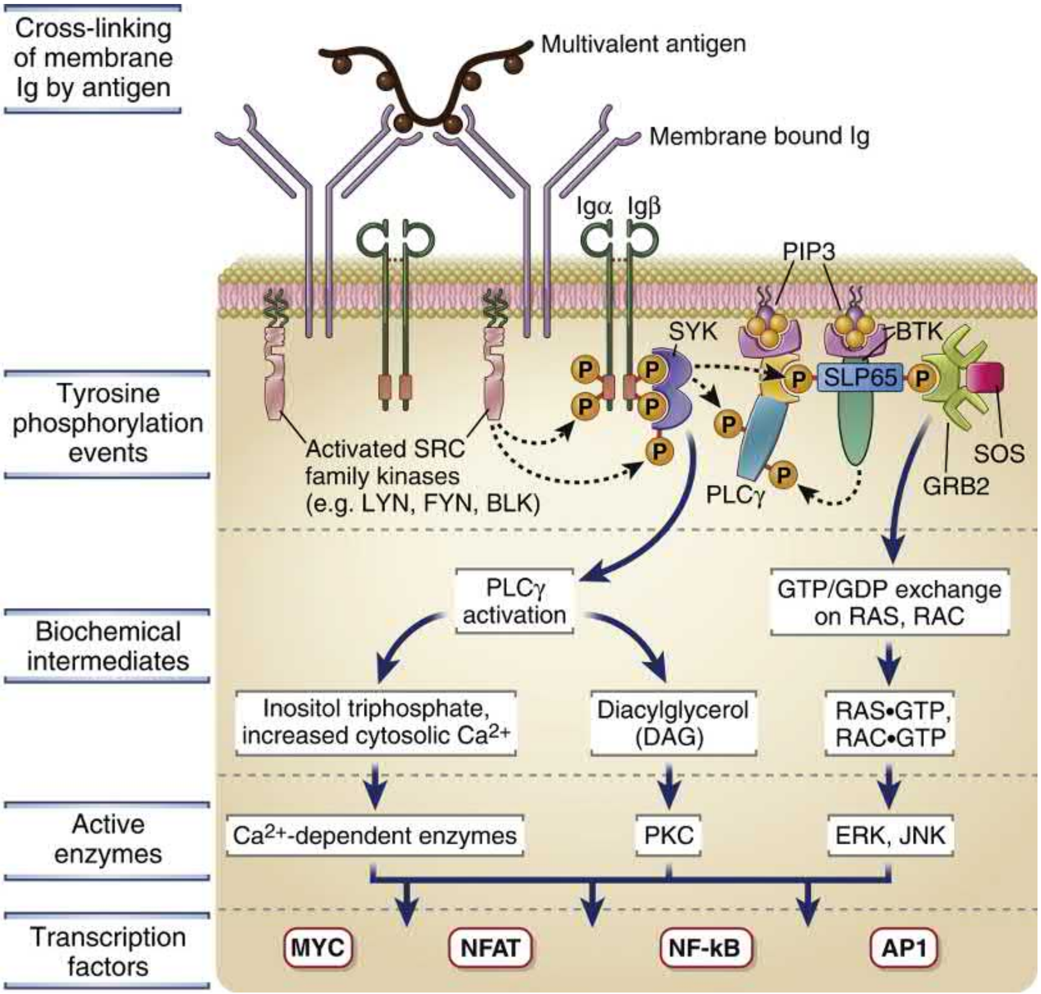
The B lymphocyte antigen receptor is a transmembrane form of an antibody molecule associated with two signaling chains. We described the structure of antibodies in detail in Chapter 5. Here we will focus on some salient features of the membrane forms of Ig and their associated proteins and discuss how they deliver signals to B cells. Because the signaling pathways are much like those in T cells, we will summarize these without great detail. As noted earlier, there are both similarities and significant differences between B and T cell antigen receptors (see Table 7.1).

Structure of the B Cell Receptor for Antigen

Membrane IgM and IgD, the antigen receptors of naive B cells, have short cytoplasmic tails consisting of only three amino acids (lysine, valine, and lysine). These tails are too small to transduce signals generated after the recognition of antigen. Ig-mediated signals are delivered by two other molecules called Igα and Igβ that are disulfide linked to one another and are expressed in B cells noncovalently associated with membrane Ig (Fig. 7.18). These proteins have functions in B cells that are similar to the functions of CD3 and ζ proteins in TCR signaling. They contain ITAMs in their cytoplasmic tails, are required for the transport of membrane Ig molecules to the cell surface, and together with membrane Ig form the **BCR complex.** The tails of Igα and Igβ are physically associated with SRC family tyrosine kinases, including LYN, FYN, and BLK. BCR complexes in class-switched B cells, including memory B cells, contain membrane Igs that may be of the IgG, IgA, or IgE class (see Chapter 12). Membrane IgG and IgE molecules have longer cytoplasmic tails that contain a conserved aspartate (or glutamate)-tyrosine-arginine-asparagine-methionine sequence called an Ig tail tyrosine (ITT) motif that is similar in sequence (and function) to a signaling motif in the cytoplasmic tail of the CD28 costimulatory receptor in T cells.

Signal Initiation by the B Cell Receptor

Signal initiation by antigens occurs by cross-linking of the BCR. Cross-linking of membrane IgM and IgD by multivalent antigens brings molecules of Igα- and Igβ-associated SRC family kinases such as LYN close to one another. The subsequent physical interaction of the kinase molecules activates these enzymes, enabling them to phosphorylate the tyrosine residues on the ITAMs of Igα and Igβ. The phosphorylation of ITAM tyrosine residues triggers all subsequent signaling events downstream of the BCR (Fig. 7.19). Cross-linked Ig receptors enter lipid rafts, where many adaptor proteins and signaling molecules are concentrated, along with SRC family tyrosine kinases such as LYN, FYN, and BLK. The phosphorylated tyrosine residues in the ITAMs of Igα and Igβ provide docking sites for the tandem SH2 domains of the SYK tyrosine kinase. SYK is homologous to ZAP70 and has similar functions in B cells as ZAP70 does in T cells. SYK is activated when it associates with phosphorylated tyrosines of ITAMs and may itself be phosphorylated on specific tyrosine residues by BCR-associated SRC family kinases, leading to further activation. Both SRC-family kinases and SYK contribute to the activation of BTK, an important tyrosine kinase in B cells. If the antigen is monovalent and incapable of cross-linking multiple Ig molecules, some signaling may nevertheless occur, but additional activation by helper T cells may be necessary to fully activate B cells, as discussed in Chapter 12.



Cross-linking of membrane Ig by antigen
Multivalent antigen
Membrane bound Ig
Igα
Igβ
PIP3
SYK
BTK
SLP65
P
Tyrosine phosphorylation events
Activated SRC family kinases (e.g. LYN, FYN, BLK)
PLCγ
GRB2
SOS
Biochemical intermediates
PLCγ activation
GTP/GDP exchange on RAS, RAC
Inositol triphosphate, increased cytosolic Ca2+
Diacylglycerol (DAG)
RAS•GTP, RAC•GTP
Active enzymes
Ca2+-dependent enzymes
PKC
ERK, JNK
Transcription factors
MYC
NFAT
NF-kB
AP1

FIGURE 7.19 Signal transduction by the B cell receptor complex.Antigen-induced cross-linking of membrane immunoglobulin *(Ig)* on B cells leads to clustering and activation of SRC family tyrosine kinases and tyrosine phosphorylation of the ITAMs in the cytoplasmic tails of the Igα and Igβ molecules. This leads to docking of SYK and subsequent tyrosine phosphorylation events as depicted. Several signaling cascades follow these events, as shown, leading to the activation of several transcription factors. These signal transduction pathways are similar to those described in T cells. *AP1,* Activator protein 1; *GDP,* guanosine diphosphate; *GTP,* guanosine

triphosphate; *ITAM,* immunoreceptor tyrosine-based activation motif; *NFAT,* nuclear factor of activated T cell; *NF-κB,* nuclear factor κB; *PLCγ1,* phospholipase C γ1; *PIP3,* phosphatidylinositol trisphosphate; *PKC,* protein kinase C.

Role of the CR2/CD21 Complement Receptor as a Coreceptor for B Cells

The activation of B cells is enhanced by signals that are provided by complement proteins and the CD21 coreceptor complex, which link innate immunity to the adaptive humoral immune response (Fig. 7.20) **.** Microbial surfaces and released polysaccharides can activate the complement system by the alternative and lectin pathways during innate immune responses, in the absence of antibodies (see Chapters 4 and 13). Proteins and other antigens that do not activate complement directly may be bound by preexisting antibodies or by antibodies produced early in the response, and these antigen-antibody complexes can activate complement by the classical pathway. Recall that complement activation results in the proteolytic cleavage of complement proteins. The key component of the system is a protein called C3, and its cleavage results in the production of a molecule called C3b that binds covalently to the microbe or antigen-antibody complex. C3b may be further degraded into a fragment called C3d, which remains bound to the microbial surface or the antigen-antibody complex. B lymphocytes express a receptor for C3d called the type 2 complement receptor (CR2, or CD21). The complex of C3d and antigen or C3d and antigen-antibody complex binds to B cells, with the membrane Ig recognizing antigen and CR2 recognizing the bound C3d (see Fig. 7.20).

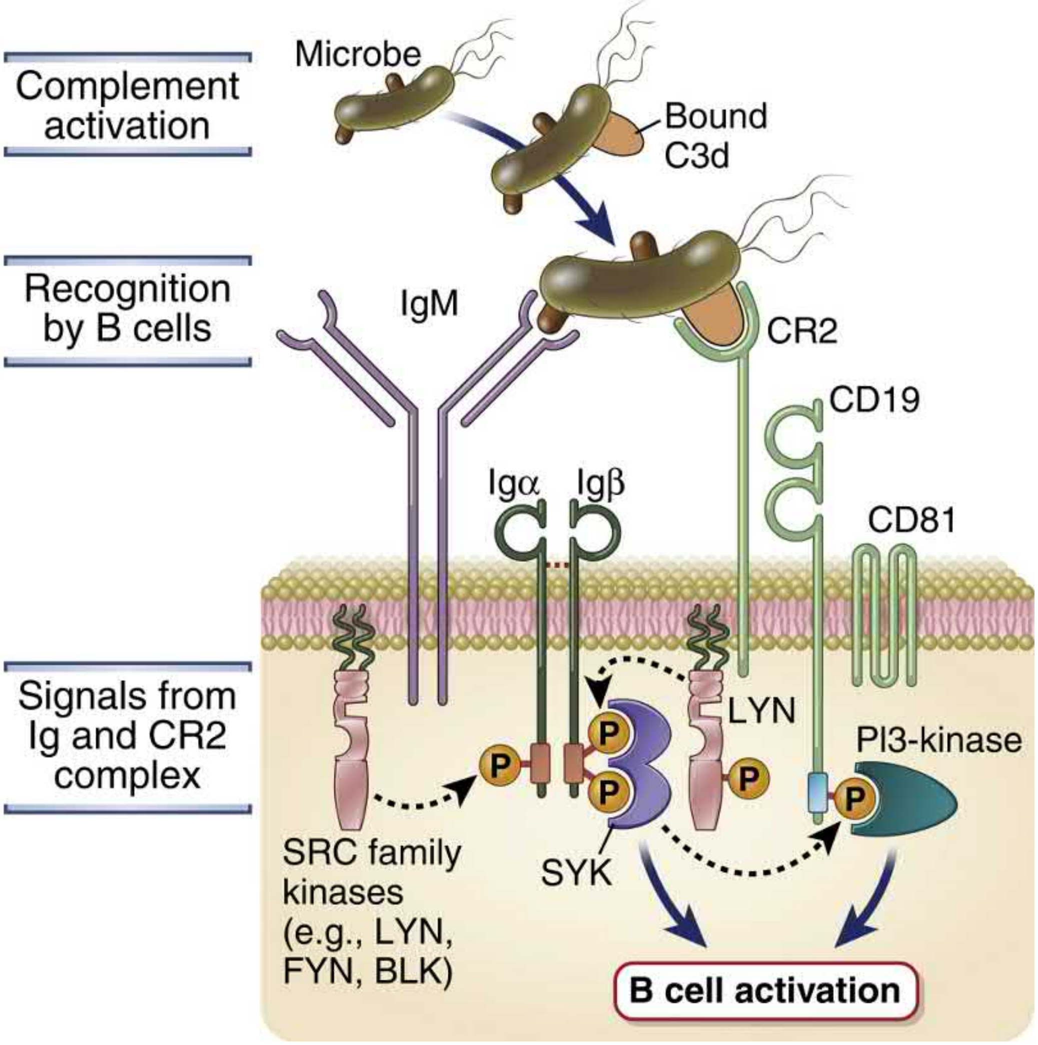

Complement activation
Microbe
Bound C3d
Recognition by B cells
IgM
CR2
CD19
Igα
Igβ
CD81
Signals from Ig and CR2 complex
LYN
PI3-kinase
P
SRC family kinases (e.g., LYN, FYN, BLK)
SYK
B cell activation

FIGURE 7.20 Role of complement in B cell activation. B cells express a complex of the CR2 complement receptor, CD19, and CD81. Microbial antigens that have bound the complement fragment C3d can simultaneously engage both the CR2 molecule and the membrane immunoglobulin *(Ig)* on the surface of a B cell. This leads to the initiation of signaling cascades from both the B cell receptor complex and the CR2 complex, because of which the response to C3d-antigen complexes is greatly enhanced compared with the response to antigen alone.

CR2 is expressed on mature B cells as a complex with two other membrane proteins, CD19 and CD81 (also called TAPA1). The CR2-CD19-CD81 complex is often called the B cell coreceptor complex because CR2 binds to antigens through attached C3d at the same time that membrane Ig binds directly to the antigen. Binding of C3d to the B cell complement receptor brings CD19 in proximity to BCR-associated kinases, and the cytoplasmic tail of CD19 rapidly becomes tyrosine phosphorylated. This leads to the activation of PI3-kinase, which generates PIP3, which in turn binds and activates BTK and PLCγ2, in a manner analogous to PDK1 activation (see Fig. 7.12) and PLCγ1 activation (see Fig. 7.15) in T cells. The net result of coreceptor activation is that the response of the antigen-stimulated B cell is greatly enhanced.

Complement proteins are not the only innate immune triggers of B cells. Other innate immune ligands, including flagellin and microbial nucleic acids, can also activate B cells via TLRs. TLR signaling has been discussed in Chapter 4, and its role in the activation of B cells will be considered in Chapter 12.

Signaling Pathways Downstream of the B Cell Receptor

After antigen binding to the BCR, SYK and other tyrosine kinases activate numerous downstream signaling pathways that are regulated by adaptor proteins (see Fig. 7.19) **.** Activated SYK phosphorylates critical tyrosine residues on adaptor proteins such as SLP65 (also called BLNK). This facilitates the recruitment to these adaptor proteins of other SH2 domain– and phosphotyrosine-binding (PTB) domain–containing enzymes, including guanine nucleotide exchange proteins that can separately activate RAS and RAC, PLCγ2, and the BTK tyrosine kinase, among others. Recruitment facilitates the activation of these downstream effectors, each generally contributing to the activation of a distinct signaling pathway.

- The **RAS-MAP kinase pathway** is activated in antigen-stimulated B cells. The GTP/GDP exchange factor SOS is recruited to the adaptor protein SLP65 through the binding of GRB2; RAS is then converted by SOS from an inactive GDP-bound form to an active GTP-bound form. Activated RAS contributes to the activation of the ERK MAP kinase pathway, as discussed earlier for T cell signaling. In a parallel fashion, the activation of the small G protein RAC may contribute to the activation of the JNK MAP kinase pathway.
- A **phosphatidylinositol-specific phospholipase C (PLC)** is activated in response to BCR signaling, and this in turn facilitates the activation of downstream signaling pathways. In B cells, the dominant isoform of PLC is the γ2 isoform, whereas T cells express the related γ1 isoform of the enzyme. PLCγ2 becomes active when it binds to the adaptor protein BLNK and is phosphorylated by BTK. As described in the context of TCR signaling, active PLC breaks down membrane PIP2 to yield soluble IP3 and leaves DAG in the plasma membrane. IP3 mobilizes calcium from intracellular stores, leading to a rapid elevation of the concentration of cytoplasmic calcium ions, which is subsequently augmented by an influx of calcium from the extracellular milieu. Calcium signaling contributes to NFAT activation in B cells as well, analogous to events described in T cells. In the presence of elevated calcium, DAG activates some isoforms of PKC (mainly PKCβ in B cells), which phosphorylate downstream proteins on serine/threonine residues.
- **PKC**β activation downstream of the BCR contributes to the activation of NF-κB in antigen-stimulated B cells. This process is similar to that in T cells triggered by PKCθ, the PKC isoform present in T cells.
- As described for T cell activation (see Fig. 7.12), the phosphorylation of specific tyrosine-containing motifs on a number of adaptors in B cells allows the recruitment and

activation of **PI3-kinase.** This enzyme facilitates critical cellular events, including cell survival, in activated B cells.

These signaling cascades ultimately lead to the activation of transcription factors that induce the expression of genes whose products are required for functional responses of B cells. Some of the transcription factors that are activated by antigen receptor–mediated signal transduction in B cells are FOS (downstream of RAS and ERK activation), JUN (downstream of RAC and JNK activation), and NF-κB (downstream of BTK, PLCγ2, and PKCβ activation). We described these earlier in the context of T cell signaling pathways. These and other transcription factors not mentioned here are involved in stimulating proliferation and differentiation of B cells (see Chapter 12).

The same signaling pathways are used by membrane IgM and IgD on naive B cells and by IgG, IgA, and IgE on B cells that have undergone isotype switching because all of these membrane Ig isotypes associate with Igα and Igβ. However, in memory B cells expressing membrane IgG or IgE, the tyrosine residue in the ITT motif is phosphorylated, recruits the GRB2 adaptor and enhances both ERK activation and Ca^{++} signaling.

The Attenuation of Immune Receptor Signaling

Activation of lymphocytes has to be tightly controlled to avoid collateral damage to host tissues during immune responses against foreign antigens. In addition, the immune system needs mechanisms that will prevent reactions against self antigens. We will describe the biology of these control mechanisms in later chapters, mainly Chapter 15. Here we discuss some of the biochemical mechanisms that limit and terminate lymphocyte activation.

Inhibitory signaling in lymphocytes is mediated primarily by inhibitory receptors and also by enzymes known as E3 ubiquitin

ligases that mark certain signaling molecules for degradation. The functional responses of all cells are regulated by a balance between stimulatory and inhibitory signals. Many inhibitory receptors recruit and activate phosphatases that counter kinase-dependent signaling events induced by receptors for antigens and costimulators (Fig. 7.21). We will describe how inhibitory receptors may function in NK cells, T cells, and B cells, and then how ubiquitin E3 ligases may attenuate signaling in lymphocytes. The biologic relevance of signal attenuation through inhibitory receptors in NK cells, T cells, and B cells is addressed in Chapters 4, 15, and 12, respectively.

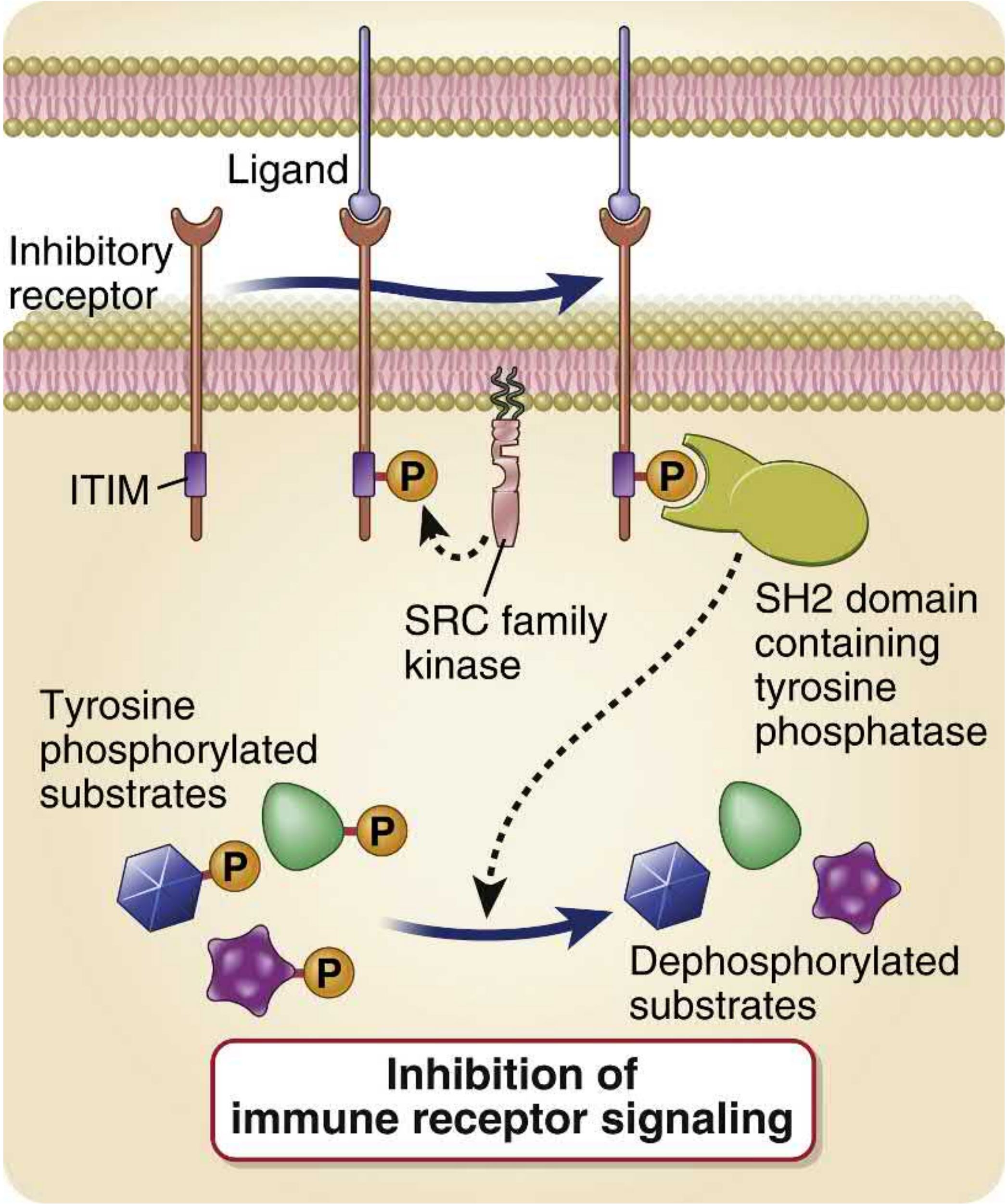

Ligand
Inhibitory receptor
ITIM
P
P
SRC family kinase
SH2 domain containing tyrosine phosphatase
Tyrosine phosphorylated substrates
P
P
P
Dephosphorylated substrates
Inhibition of immune receptor signaling

FIGURE 7.21 Inhibitory signaling in lymphocytes. A schematic depiction is provided of an inhibitory receptor with an extracellular ligand-binding domain and a cytosolic immunoreceptor tyrosine-based inhibition motif *(ITIM).* Ligand binding results in phosphorylation of the ITIM tyrosine by a SRC family kinase, followed by recruitment of an SH2 domain–containing tyrosine phosphatase that can remove phosphates from signaling

intermediates and thus attenuate immune receptor signaling.

Inhibitory Receptors of Natural Killer Cells, B Cells, and T Cells

Inhibitory receptors play key roles in NK cells, T cells, and B cells and in other cells of innate immunity. Most but not all inhibitory receptors in the immune system contain ITIMs in their cytoplasmic tails that can recruit SH2 domain–containing phosphatases and thus attenuate signaling in a broadly similar manner (see Fig. 7.21). Tyrosine residues in the ITIMs of these receptors can be phosphorylated by SRC family kinases linked to lymphocyte activation and, as described earlier, recruit SH2 domain–containing tyrosine phosphatases such as SHP1 and SHP2 and SH2 domain–containing inositol phosphatase SHIP. SHP1 and SHP2 attenuate tyrosine kinase–initiated signaling from activating receptors in NK cells and from the BCR and TCR in B and T cells, respectively. SHIP removes phosphate moieties from PIP3, as described earlier, and thus inhibits PI3-kinase activity in lymphocytes, NK cells, and innate immune cells.

In NK cells, inhibitory receptors called **killer cell immunoglobulin receptors (KIRs)** (see Chapter 4) contain extracellular Ig domains that can recognize class I human leukocyte antigen (HLA) molecules, and a subset of these receptors contains cytosolic ITIMs. The CD94/NKG2A inhibitory receptor binds to an atypical class I MHC molecule called HLA-E, and the NKG2A chain of this dimer contains cytosolic ITIMs.

The major inhibitory receptors of T cells are proteins of the CD28 family. They are also called coinhibitors, to contrast them with costimulators. One of these, **CTLA-4** (CD152), has a higher affinity than CD28 for B7 proteins and is a competitive inhibitor of B7-CD28 interactions. It inhibits immune responses mainly by out-competing an activating receptor, CD28, for its ligands, the B7 costimulators, and by removing B7 molecules from APCs. It is involved in the maintenance of unresponsiveness (tolerance) to self antigens and is discussed in this context in Chapter 15. Another inhibitory receptor of the same family is **PD-1,** and this is also discussed in Chapter 15. PD-1 contains cytosolic ITIMs and ITSMs that both contribute to inhibitory signaling in T cells. The ITSM, when phosphorylated, recruits the tyrosine phosphatase SHP2, which blocks T cell activation mediated by the TCR complex and CD28.

FcγRIIB is an important attenuator of signaling in activated B cells, DCs, and macrophages. It can bind IgG-containing immune complexes through extracellular Ig domains and primarily recruits SHIP and antagonizes PI3-kinase signaling. This receptor dampens B cell activation after antibodies are produced and will be discussed in more detail in Chapter 12.

Ubiquitin-Dependent Degradation of Signaling Proteins

One of the major ways of degrading cytosolic and nuclear proteins involves the covalent attachment of ubiquitin residues to these proteins. Although ubiquitination of proteins is frequently linked to the degradation of these proteins in proteasomes, proteins can be ubiquitinated in a number of ways, each form of ubiquitination serving a different function. In the context of signal transduction, two distinct types of ubiquitination mediate signal attenuation on the one hand and signal generation on the other.

Ubiquitination was briefly discussed in Chapter 6 in the context of the class I MHC pathway of antigen processing. Ubiquitin is a 76 amino acid protein that is activated in an ATP-dependent fashion by an E1 enzyme, then transferred to an E2 enzyme, which then covalently attaches the activated ubiquitin to lysine residues on specific substrates that are recognized by specific E3 ubiquitin ligases. In many cases, after the C terminus of a ubiquitin moiety is covalently linked to a lysine residue on a target protein, the C-terminal ends of subsequent ubiquitin moieties may be covalently attached to lysine residues on the preceding ubiquitin to generate a polyubiquitin chain. The shape of the polyubiquitin chain is different depending on which specific lysine residue on the preceding ubiquitin molecule in the chain is the site for covalent binding of the next ubiquitin molecule, and the shape of the ubiquitin chain has important functional consequences. If lysine in position 48 of the first ubiquitin moiety forms an isopeptide bond with the C terminus of the next ubiquitin and so on, a lysine-48 type of ubiquitin chain will be generated that can be recognized by the proteasomal cap and the ubiquitinated protein will be targeted for degradation in the proteasome. Some E3 ligases generate a different type of polyubiquitin chain, which does not target proteins for degradation but instead generates a structure for latching the marked proteins onto other specific proteins; this is important in NF-κB signaling, as discussed later. For some functions, in particular targeting membrane proteins to lysosomes rather than to proteasomes, only a single ubiquitin moiety may need to be attached to a protein target.

Several E3 ligases are found in T cells, some of which are involved in signal activation and others in signal attenuation. The prototype of E3 ligases involved in terminating T cell responses is CBL-b, but several others serve similar functions. Recruitment of CBL-b to the TCR complex and associated adaptor proteins leads to the monoubiquitination, endocytosis, and lysosomal degradation of the TCR complex, and this may be a mechanism for the attenuation of TCR signaling (Fig. 7.22). CD28 signals block the inhibitory activity of CBL-b, and this is one mechanism by which costimulation augments TCR signals. In knockout mice lacking Cbl-b, the T cells respond to antigen even without CD28-mediated costimulation and produce abnormally high amounts of IL-2. These mice develop autoimmunity as a result of the excessive activation of their T cells. There is some evidence that antigens that shut off immune responses (so-called tolerogenic antigens, such as self antigens) activate in T cells ubiquitin ligases that degrade essential signaling proteins, and this is a mechanism of antigen-induced unresponsiveness called anergy (see Chapter 15).

Cytokine Receptors and Signaling

Cytokines, the secreted messenger molecules of the immune system, have been mentioned in previous chapters and will be throughout the book. Here we will describe receptors for cytokines and their mechanisms of signaling.

All cytokine receptors consist of one or more transmembrane proteins whose extracellular portions are responsible for cytokine binding and whose cytoplasmic portions are responsible for initiation of intracellular signaling pathways. For most cytokine receptors, these signaling pathways are activated by ligand-induced receptor clustering, bringing together the cytoplasmic portions of two or more receptor molecules and thus inducing the activity of unique non-receptor tyrosine kinases. In the case of the TNF receptor family of cytokine receptors, preformed receptor trimers undergo a conformational change after contacting their cognate trimeric ligands.

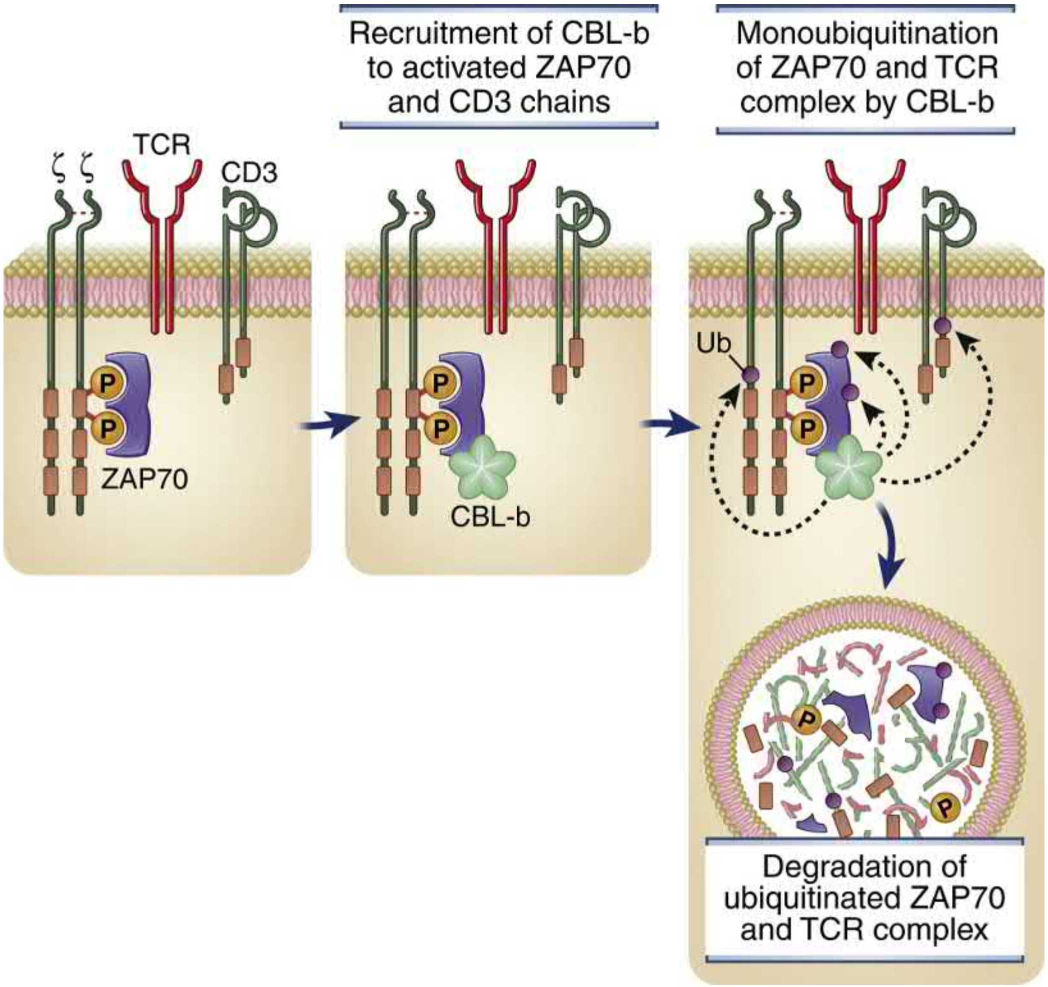

Recruitment of CBL-b to activated ZAP70 and CD3 chains
Monoubiquitination of ZAP70 and TCR complex by CBL-b
ζ ζ
TCR
CD3
P
P
ZAP70
CBL-b
Ub
Degradation of ubiquitinated ZAP70 and TCR complex

FIGURE 7.22 Role of the ubiquitin ligase CBL-b in terminating T cell responses.CBL-b is recruited to the T cell receptor *(TCR)* complex, where it facilitates the ubiquitination of CD3, ZAP70, and other proteins of the TCR complex. These proteins are targeted for proteolytic degradation in lysosomes and other organelles *(not shown).*

Classes of Cytokine Receptors

The most widely used classification of cytokine receptors is based on structural homologies of the extracellular cytokine-binding domains and shared intracellular signaling mechanisms (Fig. 7.23). Signaling mechanisms used by individual families are considered in the section that follows. The TGF-β family is one widely studied family of growth factors not included in the classification in Fig. 7.23, and its signaling mechanism is discussed later.

Type I Cytokine Receptors (Hematopoietin Receptor Family)

Type I cytokine receptors are dimers or trimers that typically consist of unique ligand-binding chains and one or more signal-transducing chains, the latter often shared by receptors for different cytokines. These chains contain one or two domains with a conserved pair of cysteine residues and a membrane proximal peptide stretch containing a tryptophan-serine-X-tryptophan-serine (WSXWS) motif, where X is any amino acid (see Fig. 7.23A). The conserved sequences of the receptors form structures that bind cytokines that have four α-helical bundles and are referred to as type I cytokines, but the specificity for individual cytokines is determined by amino acid residues that vary from one receptor to another. This receptor family can be divided into subgroups based on structural homologies or the use of shared signaling polypeptides (see Fig. 7.23B). One group, which includes receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, contains a signaling component called the common γ chain (γc, or CD132). Within this subgroup, some receptors share one of two β chain subunits (CD122 or CD131) and some lack a β chain. Another subgroup of type I cytokine receptors, including those for IL-6, IL-11, and IL-27, use the gp130 signaling chain. All the type I cytokine receptors engage JAK-STAT signaling pathways, as discussed later.

Type II Cytokine Receptors (Interferon Receptor Family)

The type II receptors are similar to type I receptors by virtue of possessing two extracellular domains with conserved cysteines, but type II receptors do not contain the WSXWS motif. All of the type II cytokine receptors, like the type I receptors, engage JAK-STAT signaling pathways. This family includes receptors for interferons (IFNs) and for IL-10, IL-20, and IL-22.

TNF Receptor Family

These receptors are part of a large family (often called the TNFR superfamily) of preformed trimers (some of which recognize membrane-associated ligands and are not considered cytokine receptors) with conserved cysteine-rich extracellular domains and shared intracellular signaling mechanisms that typically stimulate gene expression, but in some cases induce apoptosis. The important receptors of this family will be discussed in other chapters in their biologic contexts; they include the TNF receptors, TNFRI and TNFRII; the CD40 protein; FAS; the lymphotoxin receptor; and the BAFF receptor family, among others. The ligands for these receptors also form trimers. Some of these ligands are membrane bound and others are secreted, soluble proteins.

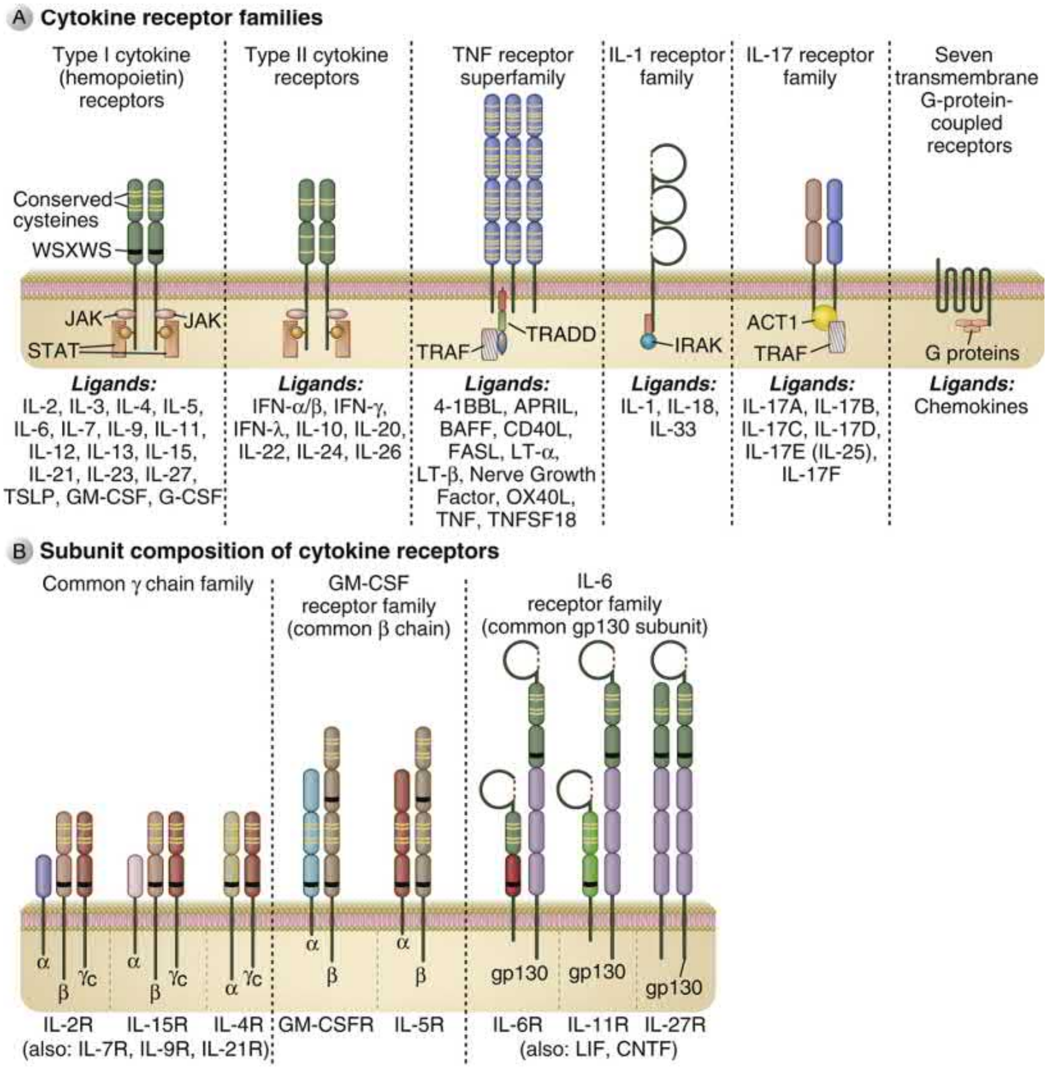

A Cytokine receptor families
Type I cytokine (hemopoietin) receptors
Type II cytokine receptors
TNF receptor superfamily
IL-1 receptor family
IL-17 receptor family
Seven transmembrane G-protein-coupled receptors
Conserved cysteines
WSXWS
JAK
JAK
STAT
TRAF
TRADD
IRAK
ACT1
TRAF
G proteins
Ligands: IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, IL-23, IL-27, TSLP, GM-CSF, G-CSF
Ligands: IFN-α/β, IFN-γ, IFN-λ, IL-10, IL-20, IL-22, IL-24, IL-26
Ligands: 4-1BBL, APRIL, BAFF, CD40L, FASL, LT-α, LT-β, Nerve Growth Factor, OX40L, TNF, TNFSF18
Ligands: IL-1, IL-18, IL-33
Ligands: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), IL-17F
Ligands: Chemokines
B Subunit composition of cytokine receptors
Common γ chain family
GM-CSF receptor family (common β chain)
IL-6 receptor family (common gp130 subunit)
α
β
γc
α
β
γc
α
γc
α
β
α
β
gp130
gp130
gp130
IL-2R
IL-15R
IL-4R
(also: IL-7R, IL-9R, IL-21R)
GM-CSFR
IL-5R
IL-6R
IL-11R
IL-27R
(also: LIF, CNTF)

FIGURE 7.23 Structure of cytokine receptors. **A,** Receptors for different cytokines are classified into families on the basis of conserved extracellular domain structures and signaling mechanisms. Representative cytokines or other ligands that bind to each receptor family are listed below the schematic drawings. **B,** Groups of cytokine receptors share identical or highly homologous subunit chains. Selected examples of cytokine receptors in each group are shown. In the common γ chain family, the IL-2 and IL-15 receptors share a β chain, CD122. In the common β chain family, the shared β chain is CD131. *G-*

CSF, Granulocyte colony-stimulating factor; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *IL,* interleukin; *TNF,* tumor necrosis factor; *WSXWS,* tryptophan-serine-X-tryptophan-serine.

Binding of the ligands to the preformed trimeric receptors typically induces a conformational change and recruits adaptor proteins to the receptor complex. These adaptors in turn recruit enzymes that include both E3 ubiquitin ligases, which mediate nondegradatory polyubiquitination, and protein kinases, which initiate downstream signaling. In the case of the TNF receptor illustrated in Fig. 7.24, the receptor recruits the adaptor protein TNF receptor–associated death domain (TRADD), and TRADD in turn can recruit proteins called TRAFs (TNF receptor–associated factors), which possess a unique type of E3 ligase activity that will be discussed in the section on NF-κB signaling. The type I TNF receptor (there are two different receptors for TNF) and FAS (CD95) can also recruit adaptors that lead to the activation of caspase-8, and these receptors can thereby induce apoptosis in certain cells.

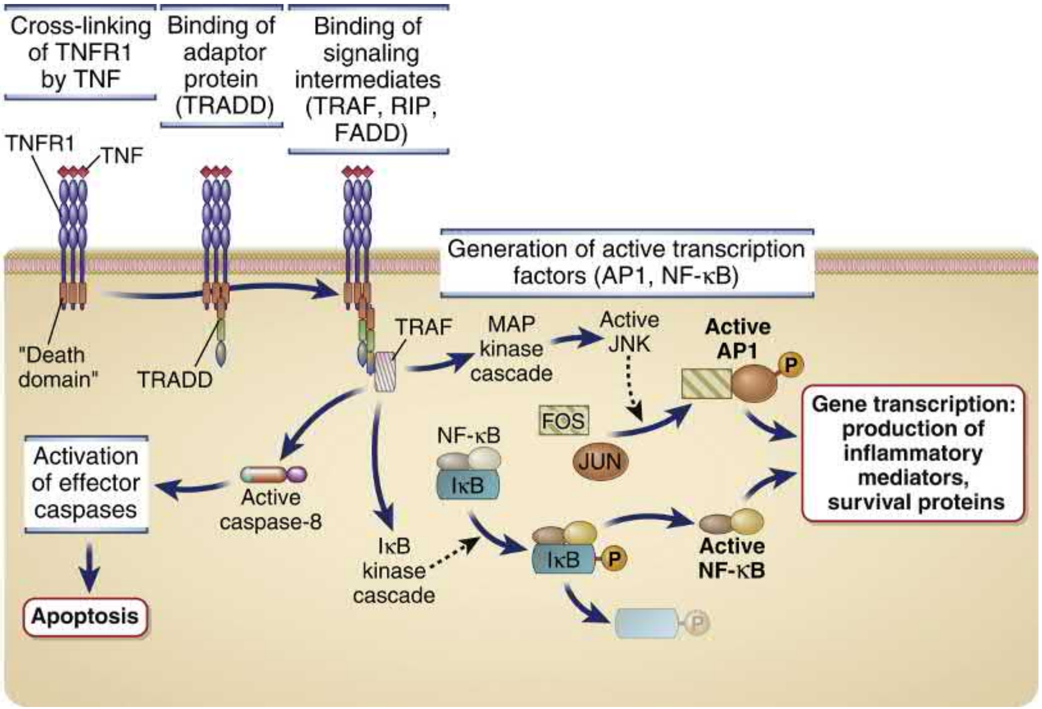

Cross-linking of TNFR1 by TNF
Binding of adaptor protein (TRADD)
Binding of signaling intermediates (TRAF, RIP, FADD)
TNFR1
TNF
Generation of active transcription factors (AP1, NF-κB)
TRAF
MAP kinase cascade
Active JNK
Active AP1
P
"Death domain"
TRADD
FOS
JUN
Gene transcription: production of inflammatory mediators, survival proteins
NF-κB
IκB
Activation of effector caspases
Active caspase-8
IκB kinase cascade
IκB
P
Active NF-κB
Apoptosis
P

FIGURE 7.24 Signaling through the TNF receptor can result in NF-κB and mitogen-activated protein kinase activation or in the induction of apoptotic death. Ligation of the type I TNF receptor results in the recruitment of an adaptor protein called TRADD, which in turn can activate TRAF molecules (E3 ubiquitin ligases) and the RIP1 kinase. Downstream consequences include the activation of the NF-κB pathway and the JNK MAP kinase pathway or the induction of apoptotic death. *MAP,* Mitogen-activated protein; *NF-κB,* nuclear factor κB; *TNF,* tumor necrosis factor. *TRAF,* TNF receptor–associated factors; *TRADD,* TNF receptor–associated death domain.

IL-1 Family

The receptors of this family share a conserved cytosolic sequence, called the Toll/IL-1 receptor (TIR) domain, and engage similar signal transduction pathways that induce new gene transcription. We discussed TLR signaling in Chapter 4. Briefly, engagement of the IL-1R or of TLRs results in receptor dimerization and the recruitment of one or more of four known TIR domain–containing adaptors to the TIR domain of the cytoplasmic tail of the receptor. The adaptors link TLRs to different members of the IL-1 receptor–associated kinase (IRAK) family. IRAKs can in turn link adaptors to TRAF6, an E3 ubiquitin ligase required for NF-κB activation. Other events downstream of TLR signaling include MAP kinase activation and the phosphorylation of IRF3 and IRF7, inducers of type I IFN gene transcription. The latter aspect of TLR signaling has been considered in the context of the antiviral state in Chapter 4. Different adaptors link TLRs to NF-κB signaling, MAP kinase activation, and the activation of IRF3. The mechanisms connecting IL-1R/TLR signaling and NF-κB activation are discussed later.

IL-17 Family

The receptors of this family are preformed oligomers that include various combinations of the IL-17R A, B, C, D, and E chains. Receptor oligomers include at least one molecule of the IL-17RA chain. Each receptor chain is a type I integral membrane protein that contains two extracellular type III fibronectin domains and an intracellular SEFIR motif, which has partial homology to the TIR motif discussed in the context of IL-1 receptor signaling. The SEFIR motif, however, does not recruit adaptors that bind to TLRs and the IL-1 receptor. This motif binds to an adaptor ACT1 that also contains a SEFIR motif and contributes to the recruitment of TRAF6 and leads to NF-κB activation. Additional cytoplasmic motifs are required for the activation of many other pathways, including the ERK pathway and the C/EBP family of transcription factors.

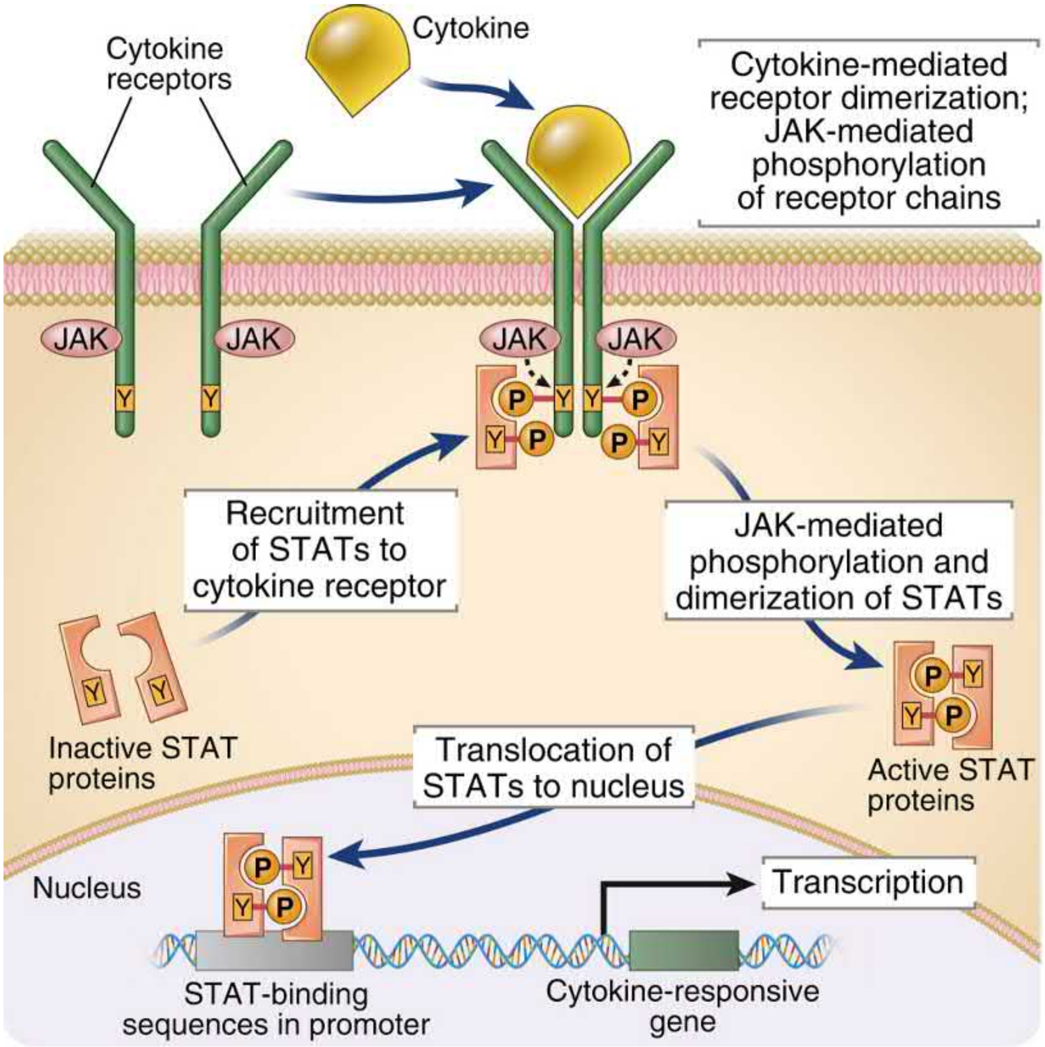

Cytokine
Cytokine receptors
Cytokine-mediated receptor dimerization; JAK-mediated phosphorylation of receptor chains
JAK
JAK
JAK
JAK
Y
P
Recruitment of STATs to cytokine receptor
JAK-mediated phosphorylation and dimerization of STATs
Inactive STAT proteins
Active STAT proteins
Translocation of STATs to nucleus
Nucleus
Transcription
STAT-binding sequences in promoter
Cytokine-responsive gene

FIGURE 7.25 JAK-STAT signaling induced by cytokines. Ligation of receptors for type I and type II cytokines results in the activation of an associated JAK tyrosine kinase, the phosphorylation of the receptor tail, and the recruitment of an SH2 domain–containing activator of transcription *(STAT)* to the receptor. The recruited STAT is activated by JAK phosphorylation, dimerizes, enters the nucleus, and turns on the expression of cytokine target genes. *JAK*, Janus kinases; *STATs*, signal transducers and activators of transcription.

There are many different cytokines of the IL-17 family, and in subsequent chapters the emphasis will largely be on the cytokines that are best characterized in the context of autoimmunity and inflammation, namely IL-17A and IL-17F. IL-17 B, C, and D remain poorly characterized, and IL-17E, also known as IL-25, drives Th2 responses. IL-17A homodimers and IL-17F homodimers engage preformed heterodimers made up of IL-17RA and IL-17RC. IL-17E/IL-25 engages a dimer containing IL-17RB and IL-17RA.

Signaling by JAKs and STATs

Cytokine receptors of the type I and type II receptor families engage signal transduction pathways that involve non-receptor tyrosine kinases called Janus kinases (JAKs) and transcription factors called signal transducers and activators of transcription (STATs). The discovery of the JAK-STAT pathways came from biochemical and genetic analyses of IFN signaling. There are four known JAKs (JAKs1 to 3 and TYK2) and seven STATs (STATs 1 to 4, 5a, 5b, and 6).

The sequence of events in the JAK-STAT signaling pathways is now well defined (Fig. 7.25). Inactive JAK enzymes are noncovalently attached to the cytoplasmic domains of type I and type II cytokine receptors. When two receptor molecules are brought together by binding of a cytokine molecule, the receptor-associated JAKs are activated and phosphorylate tyrosine residues in the cytoplasmic portions of the clustered receptors. Some of these phosphotyrosine moieties of the receptors are then recognized by and bind SH2 domains of monomeric cytosolic STAT proteins. The STAT proteins are thus brought close to JAKs and are phosphorylated by these receptor-associated kinases. The SH2 domain of one STAT monomer is able to bind to a phosphotyrosine residue on an adjacent STAT protein. The STAT dimers that are generated migrate to the nucleus, where they bind to specific DNA sequences in the promoter regions of cytokine-responsive genes and activate gene transcription.

An intriguing question is how the specificity of responses to many different cytokines is achieved, given the limited numbers of JAKs and STATs used by the large number of cytokine receptors. The likely answer is that unique amino acid sequences in the different cytokine receptors provide the scaffold for specifically binding, and thereby activating, different combinations of JAKs and STATs. The SH2 domains of different STAT proteins selectively bind to phosphotyrosines and flanking residues of different cytokine receptors. This is largely responsible for the activation of particular STATs by various cytokine receptors and therefore for the specificity of cytokine signaling. Several type I and type II cytokine receptors are heterodimers of two different polypeptide chains, each of which binds a different JAK. Furthermore, two different STATs may heterodimerize on phosphorylation. Thus, there is a significant amount of combinatorial diversity in the signaling that can be generated from a limited number of JAK and STAT proteins. The subset of type I cytokine receptors that use the common γ chain all use the JAK3 kinase for signaling. JAK3 is the only JAK kinase that is not expressed ubiquitously. Its expression is largely restricted to immune cells, and it is only activated by γc-containing receptors. Type I cytokine receptors of the IL-6 family use JAK2 to activate STAT3. A number of other cytokines also activate STAT3.

Several JAKs and STATs are relevant to human disease and are targets of therapeutic agents. Gain-of-function mutations in JAK2 are the cause of myelodysplastic syndrome with aplastic anemia and polycythemia vera. Mutations affecting the γc chain or, less frequently, JAK3, cause severe combined immunodeficiency (see Chapter 21). Dominant-negative mutations in STAT3 cause an immunodeficiency disease due to defects in Th17 responses. Activating mutations in STAT3 are characteristic features of large granular lymphocytic leukemias that are malignant proliferation of cells of the NK cell or the CD8^{+} T cell lineages. The elucidation of JAK-STAT signaling has also led to the development of novel therapeutic agents targeting these pathways. Small molecule JAK antagonists are approved for the treatment of acute myeloid leukemia and some other myeloid malignancies and also for certain chronic inflammatory diseases, including rheumatoid arthritis and psoriasis.

Cytokines activate signaling pathways and transcription factors in addition to the JAKs and STATs. For instance, the IL-2 receptor β chain activates RAS-dependent MAP kinase pathways that may be involved in gene transcription and growth stimulation. Other cytokine receptors may similarly activate other signaling pathways in concert with the JAK-STAT pathways to elicit biologic responses to the cytokines. T cell proliferation, triggered to a considerable degree by cytokines such as IL-2, is targeted by some immunosuppressive small molecules. An important downstream protein kinase that regulates protein translation and cell growth in many cell types, including dividing T cells, is mTOR. It is, as its name implies, inhibited by rapamycin, a clinically used immunosuppressive drug.

Several mechanisms of negative regulation of JAK-STAT pathways have been identified. Proteins called suppressors of cytokine signaling (SOCS) serve as adaptors for multisubunit E3 ligase activity. They can bind to activated STATs and JAKs, and the tightly associated E3 ligases ubiquitinate the JAKs and STATs, targeting them for proteasomal degradation. SOCS protein levels can be regulated by TLR ligands, by cytokines themselves, and by other stimuli. In this way, SOCS proteins serve as negative feedback regulators of the cytokine-mediated activation of cells. Other inhibitors of JAK-STAT signaling include tyrosine phosphatases, such as SHP1 and SHP2, which can dephosphorylate and therefore deactivate JAK molecules. Another family of inhibitory proteins, called protein inhibitors of activated STAT (PIAS), binds phosphorylated STATs and prevents their interaction with DNA. It is now known that PIAS proteins also interact with and block the function of other transcription factors associated with cytokine signaling, including NF-κB and SMADs (transcription factors downstream of members of the TGF-β receptor family).

Pathways of NF-κB Activation

NF-κB refers to a group of structurally related transcription factors that play a central role in inflammation, lymphocyte activation, cell survival, and the formation of secondary lymphoid organs. NF-κB family members are important players in lymphocyte development and in the pathogenesis of many cancers, including malignant neoplasms derived from activated lymphocytes. NF-κB is prominently activated by cytokines of the IL-1, TNF, and IL-17 families, and is also induced downstream of TLR stimulation and antigen recognition. It is discussed here as the prototype of a transcription factor with fundamental roles in innate and adaptive immunity.

There are five NF-κB proteins. The domain that is common to all NF-κB proteins is a DNA-binding domain called a REL homology domain. For a transcription factor to be active, it must both bind DNA and contain an activation domain that can facilitate transcriptional initiation. Three NF-κB proteins have both REL homology domains and activation domains. These are p65/REL-A, REL-B, and c-REL. Two proteins, NF-κB1/p50 and NF-κB2/p52, contain a DNA-binding REL homology domain but lack activation domains. NF-κB1 typically forms active heterodimers with p65/REL-A or with c-REL, and NF-κB2 forms heterodimers with REL-B.

There are two pathways of NF-κB activation, called the canonical and noncanonical pathways (Fig. 7.26) **.** Most stimuli that activate NF-κB do so by inducing the canonical pathway (hence its name). This pathway is activated by a number of receptors that drive inflammation, such as TLRs, IL-1R, and some members of the TNFR family, such as TNFRI. It is also activated in the context of lymphocyte activation by the BCR and TCR. The canonical pathway results in the nuclear localization of transcriptionally active heterodimers of NF-κB1 with p65/REL-A or with c-REL. NF-κB1/p50-containing heterodimers normally reside in the cytosol bound to an inhibitor of NF-κB called IκBα, and they cannot access the nucleus in nonactivated cells (see Fig. 7.26). The canonical NF-κB pathway induces the tagging and degradation of IκBα, allowing the unfettered heterodimeric NF-κB1 containing transcription factor to migrate into the nucleus. Two very different types of polyubiquitination events are required for canonical NF-κB activation. There are a few common steps in the canonical pathway that apply to all upstream signal inputs.

- Upstream signaling leads to the activation of a unique type of ubiquitin E3 ligase that can add a lysine-63 type of ubiquitin chain to a protein called NEMO or IKKγ that is a noncatalytic subunit of a trimeric enzyme complex called the IκB kinase (IKK) complex. This complex contains two other subunits called IKKα and IKKβ, both of which have the potential to be catalytically active serine/threonine kinases. Ubiquitination of NEMO allows IKKβ to be activated by an upstream kinase.
- Active IKKβ phosphorylates the inhibitory protein bound to NF-κB, IκBα, on two specific serine residues, and thus tags this protein for lysine-48 ubiquitination.
- Polyubiquitinated IκBα is targeted for degradation in the proteasome, and the canonical NF-κB heterodimer is then free to enter the nucleus (see Fig. 7.26).

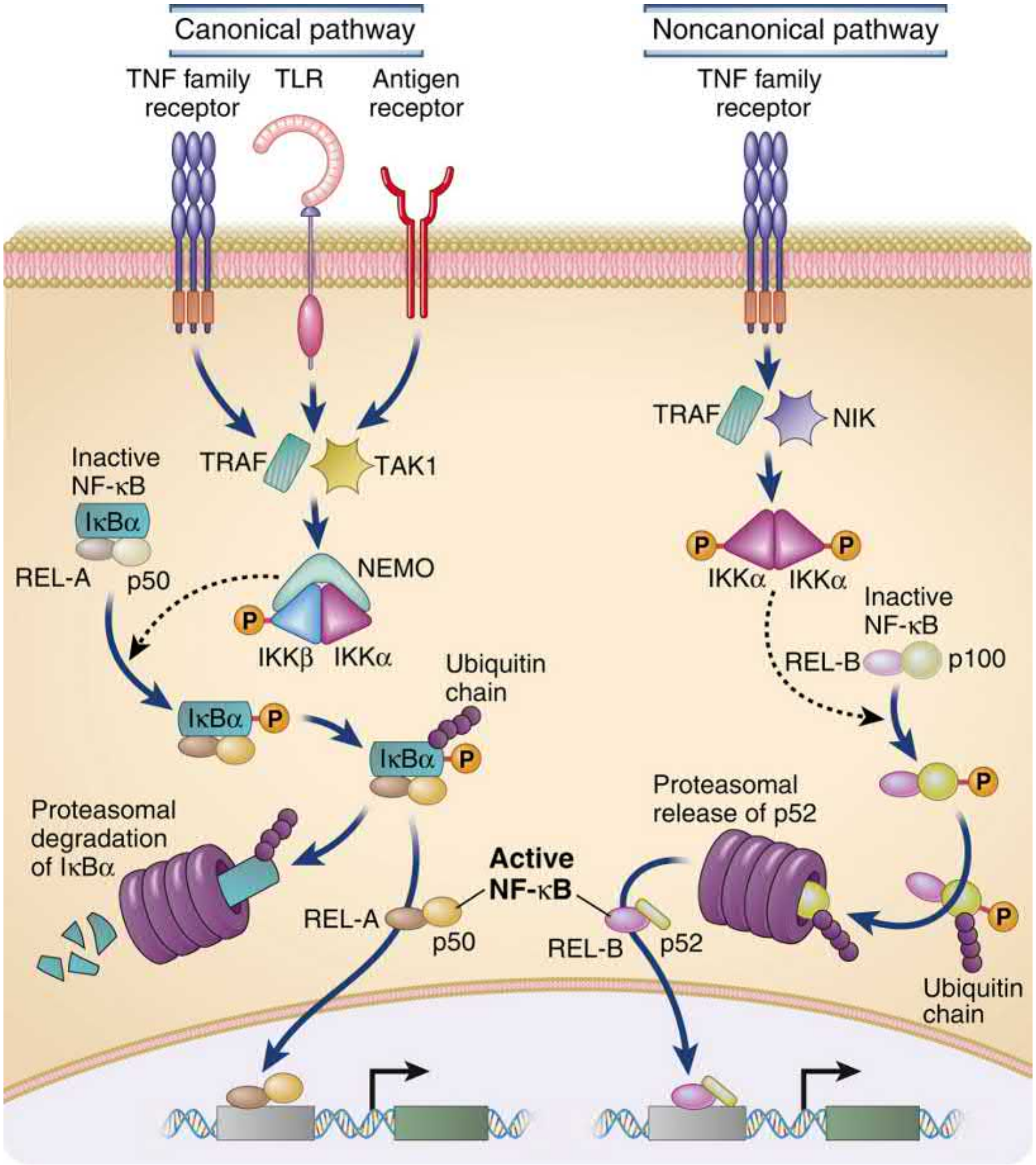

Canonical pathway
TNF family receptor
TLR
Antigen receptor
Noncanonical pathway
TNF family receptor
TRAF
TAK1
TRAF
NIK
Inactive NF-κB
IκBα
REL-A
p50
NEMO
P
IKKβ
IKKα
IKKα
IKKα
Inactive NF-κB
REL-B
p100
Ubiquitin chain
IκBα
P
Proteasomal degradation of IκBα
Active NF-κB
REL-A
p50
REL-B
p52
Proteasomal release of p52
Ubiquitin chain

FIGURE 7.26 The canonical and noncanonical NF-κB pathways. The canonical pathway is depicted on the *left.* TNF family receptors, TLRs, and antigen receptors activate or induce an E3 ligase that can polyubiquitinate NEMO/IKKγ, a component of the *IκB* kinase (IKK) complex, forming lysine-63–linked ubiquitin chains. Degradation of IκBα leads to the entry of active NF-κB into the nucleus. Antigen receptors activate specific PKCs, which in turn activate the CARMA1/BCL-10/MALT1 complex (not shown) in

order to activate IKK. The noncanonical pathway is depicted on the right. In this pathway, most prominently activated downstream of the lymphotoxin β receptor and the BAFF receptor, TRAFs are activated downstream of these receptors, also in a lysine-63 ubiquitination-dependent manner *(not shown)*. Other details are in the text. *NF-κB,* Nuclear factor κB; *PKC,* protein kinase C; *TLR,* Toll-like receptor; *TNF,* tumor necrosis factor; *TRADD,* TNF receptor–associated death domain; *TRAF,* TNF receptor–associated factor.

We discussed earlier how TCR and BCR signaling contributes to the activation of PKCθ and PKCβ, respectively. These PKCs can phosphorylate a protein called CARMA1 that forms a complex with two proteins called BCL-10 and MALT1. The CARMA1/MALT1/BCL-10 complex can contribute to the activation of a lysine-63 type of ubiquitin E3 ligase called TRAF6. Active TRAF6 can activate TAK1 and also add a lysine-63 ubiquitin chain to NEMO, thus facilitating the activation of IKKβ. TLRs, IL-17R, and the IL-1R also activate TRAF6 to initiate IKK activation. Many members of the TNF receptor family, including the TNF receptor and CD40, can activate canonical NF-κB signaling through the activation of other TRAF proteins such as TRAF2, TRAF3, and TRAF5.

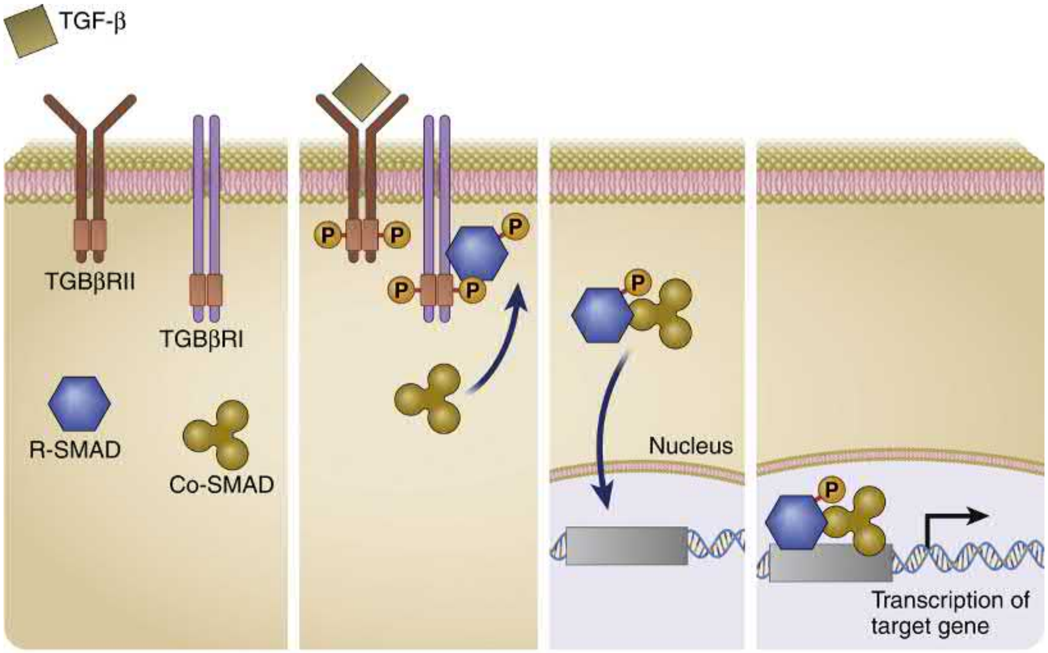


FIGURE 7.27 The TGF-β signaling pathway.Processed transforming growth factor-β (TGF-β) dimers bind to the TGβRII receptor, which is a dimeric receptor serine/threonine kinase. TGFβRII is activated and then associates with and phosphorylates the TGFβRI dimer, also a receptor serine/threonine kinase. Catalytically active TGFβRI then phosphorylates a receptor-regulated SMAD transcription factor (an R-SMAD such as SMAD2 or SMAD3) that resides in the cytosol and which then forms a heterodimer with a Co-SMAD, such as SMAD4. This SMAD heterodimer enters the nucleus and induces the transcription of target genes.

A separate NF-κB signaling pathway, called the noncanonical pathway, processes a precursor protein called p100 into p52, thus allowing heterodimers of NF-κB2/p52 and its partner REL-B to enter the nucleus. In nonactivated cells, the p100 precursor is bound to REL-B, and the p100/REL-B complex is unable to enter the nucleus until p100 is converted to p52. This pathway is activated downstream of a few TNFR family signaling receptors, most notably the lymphotoxin-β receptor (LTβR) that drives lymphoid organogenesis and the BAFF receptor (BAFFR) that facilitates B cell survival. Receptors such as LTβR and BAFFR that induce the noncanonical NF-κB pathway also use TRAFs to activate a kinase called NIK that in turn activates an IKK-like complex that contains IKKα homodimers. This leads to the phosphorylation of p100, marking it for ubiquitination and degradation in the cytosol, and leads to the generation of noncanonical p52/REL-B NF-κB complexes that can migrate into the nucleus (see Fig. 7.26).

TGF-β Signaling

Transforming growth factor-β1 (TGF-β1) is a growth factor of importance in T and B cell biology. It is one member of the widely studied TGF-β superfamily of structurally related growth factors that play crucial roles in numerous aspects of invertebrate and vertebrate development as well as in cellular transformation in cancer. During T cell differentiation, TGF-β participates in regulatory T cell development (see Chapter 15) and the development of Th17 cells (see Chapter 10). In humoral immunity, TGF-β is important for class switching to IgA (see Chapter 12).

Newly synthesized TGF-β is cleaved in the Golgi into two fragments, a C-terminal peptide that dimerizes to form the inactive cytokine and an N-terminal pro-domain, called latency-associated peptide, that also dimerizes and remains bound to the inactive TGF-β dimer. After secretion, the latency-associated peptide dimer, still bound to the inactive TGF-β dimer, associates covalently (forming disulfide bonds) with a specific protein on the extracellular matrix forming what is known as the latent complex. α_V integrins on another cell bind to the latency-associated peptide and the activated integrin then exerts physical force, disrupting the latent complex and leading to the release of active TGF-β dimers. TGF-β dimers then bind to the dimeric TGFβRII receptor protein on an adjacent cell surface.

Mammalian cells contain seven different TGF-β family type I receptors and five different type II receptors. Both the TGFβRI and the TGFβRII proteins are transmembrane protein dimers whose extracellular domains bind TGF-β and whose cytosolic tails contain serine/threonine kinase domains. After TGFβRII has been ligated, it is catalytically activated and phosphorylates and activates TGFβRI. Catalytically active TGFβRI then phosphorylates a cytosolic receptor-regulated SMAD family transcription factor or R-SMAD (such as SMAD2 or SMAD3), which then acquires a high affinity for a co-SMAD such as SMAD4. The R-SMAD–co-SMAD complex then goes to the nucleus and induces transcription of TGF-β target genes (Fig 7.27). Depending on the cell type, these target genes differ. In the induction of peripheral or induced regulatory T cells for instance (see Chapter 15), TGF-β signaling induces the expression of the FOXP3 transcription factor, which is essential for the development and inhibitory function of these regulatory T cells.

Summary

- Signaling receptors, typically located on the cell surface, generally initiate signaling in the cytosol, followed by a nuclear phase during which gene expression is altered.
- Many different types of signaling receptors contribute to innate and adaptive immunity, the most prominent category being immune receptors that belong to a receptor family in which non-receptor tyrosine kinases phosphorylate tyrosine-containing immunoreceptor tyrosine-based activation motif (ITAM) on the cytoplasmic tails of proteins in the receptor complex.
- Some of the other types of receptors of interest in immunology include those of the receptor tyrosine kinase family, nuclear receptors, heterotrimeric G protein–coupled serpentine receptors, and receptors of the Notch family.
- Antigen receptors on T and B cells, as well as immunoglobulin (Ig) Fc receptors, are members of the immune receptor family.

- Antigen receptors can produce widely varying outputs, depending on the affinity and valency of the antigen that can recruit different numbers of ITAMs.
- Coreceptors, such as CD4 or CD8 on T cells and CD21 (CR2) on B cells, enhance signaling from antigen receptors. Coreceptors bind to the same antigen complex that is being recognized by the antigen receptor.
- Signaling from antigen receptors can be attenuated by inhibitory receptors such as CD22 and PD-1, which contain cytosolic immunoreceptor tyrosine-based inhibition motifs (ITIMs) and sometimes immunoreceptor tyrosine-based switch motifs (ITSMs).
- The T cell receptor (TCR) complex is made up of the TCR α and β chains that contribute to antigen recognition and the ITAM-containing signaling chains CD3 γ, δ, and ε and the ζ homodimer. The CD3 chains each contain one ITAM, whereas each ζ chain contains three ITAMs.
- TCR ligation results in tyrosine phosphorylation of CD3 and ζ ITAMs by SRC family kinases and the recruitment of ZAP70 to the phospho-ITAMs, with each SH2 domain of ZAP70 binding to one phosphorylated tyrosine of the ITAM. Activated ZAP70 phosphorylates tyrosine residues on adaptors, and downstream enzymes are recruited to the signalosome.
- Enzymes that mediate the exchange of GTP for GDP on small G proteins such as RAS and RAC help initiate mitogen-activated protein (MAP) kinase pathways. These pathways lead to the induction or activation of transcription factors such as JUN and FOS, components of the AP1 transcription factor.
- Activation of PLCγ1 leads to the release of IP3 from PIP2, and IP3 induces release of calcium from intracellular stores. Depletion of calcium from intracellular stores facilitates the opening of the calcium release–activated calcium (CRAC) channel, a store-operated channel on the cell surface that maintains the raised intracellular calcium levels. Calcium

binds to calmodulin and activates downstream proteins, including calcineurin, a phosphatase that facilitates the entry of the nuclear factor of activated T cell (NFAT) transcription factor into the nucleus.

- DAG is generated in the membrane when PLCγ1 releases IP3 from PIP2. DAG can activate PKCθ, which, among other things, can contribute to NF-κB activation.
- A lipid kinase called PI3-kinase converts PIP2 to PIP3. PIP3 can recruit and activate pleckstrin homology (PH) domain–containing proteins to the plasma membrane. PIP3 activates ITK in T cells and BTK in B cells. It activates PDK1, a kinase that can phosphorylate a downstream kinase called AKT that mediates cell survival.
- Costimulatory receptors initiate signaling separately from antigen receptors, and signaling outputs from antigen receptors and costimulatory receptors synergize in the nucleus. The major costimulatory receptor in T cells is CD28.
- The BCR is made up of membrane-bound Ig and an associated disulfide-linked Igα and Igβ heterodimer. Both Igα and Igβ contain ITAMs in their cytoplasmic tails. Signaling pathways linked to the BCR are broadly similar to signaling pathways downstream of the TCR.
- Attenuation of immune receptor signaling in B cells, T cells, and NK cells, among others, is mediated by inhibitory receptors that frequently contain inhibitory tyrosine-containing motifs or ITIMs in their cytoplasmic tails and recruit phosphatases.
- Another important mechanism of signal attenuation involves the ubiquitination of signaling proteins by E3 ubiquitin ligases, which tags these proteins for intracellular degradation.
- Cytokine receptors can be divided into categories based on structural features and mechanisms of signaling.
- Many cytokine receptors use non-receptor tyrosine kinases called JAKs to phosphorylate transcription factors called

STATs, which dimerize after phosphorylation, translocate to the nucleus, and induce transcription of target genes.

- Some cytokine receptors such as those of the interleukin-1 (IL-1), IL-17, and tumor necrosis factor (TNF) receptor families activate either canonical or noncanonical NF-κB signaling.
- Canonical NF-κB signaling is activated downstream of many receptors, including TNF receptor family cytokine receptors, Toll-like receptors (TLRs) and IL-1R family members, and antigen receptors. The pathway involves activation of IKKβ in the IKK complex, phosphorylation of the IκBα inhibitor by activated IKKβ, ubiquitination and proteasomal degradation of IκBα, and transport of NF-κB to the nucleus.
- Transforming growth factor-β (TGF-β) binds to TGF-βRII, which phosphorylates and activates TGF-βRI, which in turn phosphorylates and activates an R-SMAD. The phosphorylated R-SMAD then dimerizes with a co-SMAD, and this dimer enters the nucleus and stimulates transcription of target genes.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

Signal Transduction in Immune Cells

Wu N, Veillette A. SLAM family receptors in normal immunity and immune pathology. *Curr Opin Immunol* . 2016;38:45–51.

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T Cell Receptor Structure and Signaling

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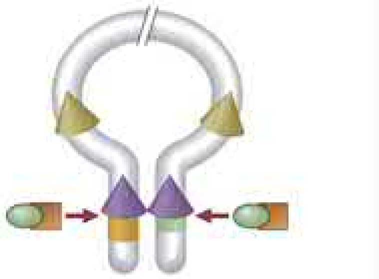
Chapter 8: Lymphocyte Development and Antigen Receptor Gene Rearrangement



Overview of Lymphocyte Development,
    Commitment to the B and T Cell Lineages and Proliferation of Progenitors,
    Role of Epigenetic Changes and MicroRNAs in Lymphocyte Development,
    Antigen Receptor Gene Rearrangement and Expression,
    Selection Processes That Shape the B and T Lymphocyte Repertoires,

Rearrangement of Antigen Receptor Genes in B and T Lymphocytes,
    Germline Organization of Immunoglobulin and T Cell Receptor Genes,
    V(D)J Recombination,
    Generation of Diversity in B and T Cells,

B Lymphocyte Development,
    Stages of B Lymphocyte Development,
    Selection of the Mature B Cell Repertoire,

T Lymphocyte Development,
    Role of the Thymus in T Cell Maturation,
    Stages of T Cell Maturation,
    Selection Processes in the Maturation of MHC-Restricted αβ T Cells,
    γδ T Lymphocytes,

Summary,

Lymphocytes express highly diverse antigen receptors that are capable of recognizing a wide variety of foreign substances. This diversity is generated during the development of mature B and T lymphocytes from precursor cells that do not express antigen receptors and cannot recognize and respond to antigens. The process by which lymphocyte progenitors in the thymus and bone marrow differentiate into mature lymphocytes that populate peripheral lymphoid tissues is called **lymphocyte development** or **lymphocyte maturation.** (The terms development and maturation are used interchangeably in this context.) Maturation is initiated by signals from cell surface receptors that have two main roles: they promote the proliferation of progenitors, and they initiate the rearrangement of antigen receptor genes, which is required for the development of B and T lymphocytes with diverse antigen specificities.

We begin this chapter by considering the process of commitment to the B and T lymphocyte lineages and discussing some common principles and mechanisms of lymphocyte development. This is followed by a description of the processes that are unique to the development of B cells and then those unique to T cells.

Overview of Lymphocyte Development

The maturation of B and T lymphocytes involves a series of events that occur in the primary (also called generative or central) lymphoid organs (Fig. 8.1). These events include the following:

- ***Commitment*** of progenitor cells to the B lymphoid or T lymphoid lineage.
- ***Proliferation*** of progenitors and immature committed cells at specific early stages of development, providing a large pool of cells that can generate useful lymphocytes.
- The ***sequential and ordered rearrangement of antigen receptor genes*** and the expression of antigen receptor proteins. (The terms rearrangement and recombination are used interchangeably.)
- ***Selection events*** that preserve cells that have produced functional antigen receptor proteins and eliminate potentially dangerous cells that strongly recognize self antigens. These selection processes during development ensure that lymphocytes that express functional receptors with useful specificities will mature and enter the peripheral immune system.
- ***Differentiation of B and T cells into functionally and phenotypically distinct subpopulations.*** B cells develop into follicular, marginal zone, and B-1 cells, and T cells develop into $CD4^+$ and $CD8^+$ αβ T lymphocytes, γδ T cells, natural killer T (NKT) cells, and mucosa-associated invariant T (MAIT) cells. The properties and functions of these different lymphocyte populations are discussed in later chapters.

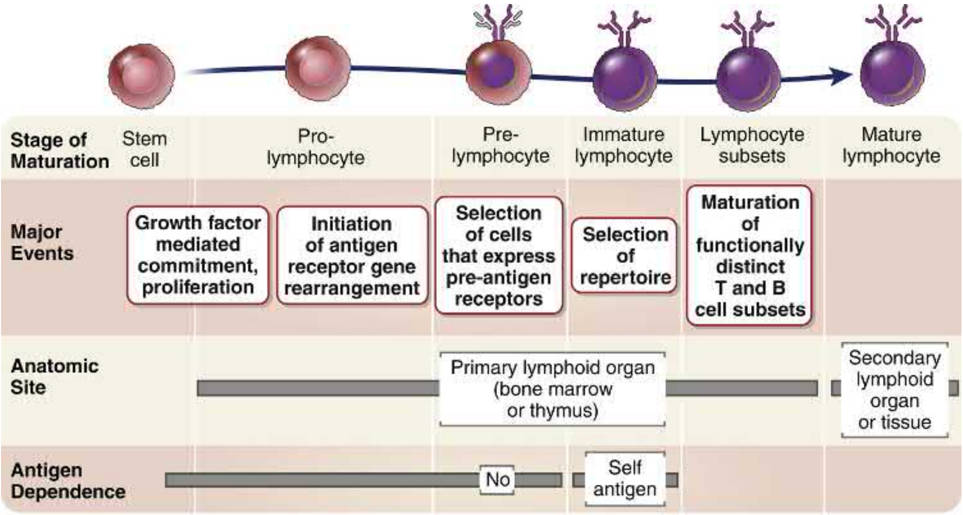

Stage of Maturation
Stem cell
Pro-lymphocyte
Pre-lymphocyte
Immature lymphocyte
Lymphocyte subsets
Mature lymphocyte
Major Events
Growth factor mediated commitment, proliferation
Initiation of antigen receptor gene rearrangement
Selection of cells that express pre-antigen receptors
Selection of repertoire
Maturation of functionally distinct T and B cell subsets
Anatomic Site
Primary lymphoid organ (bone marrow or thymus)
Secondary lymphoid organ or tissue
Antigen Dependence
No
Self antigen

FIGURE 8.1 Stages of lymphocyte maturation.Development of both B and T lymphocytes involves the sequence of maturational stages shown. B cell maturation is illustrated, but the basic stages of T cell maturation are similar.

Commitment to the B and T Cell Lineages and Proliferation of Progenitors

Multipotent stem cells in the fetal liver and bone marrow, known as hematopoietic stem cells (HSCs), give rise to all lineages of blood cells, including lymphocytes (see Chapter 2) . HSCs mature into common lymphoid progenitors that can give rise to B cells, T cells, NK cells, and innate lymphoid cells (Fig. 8.2). The maturation of B cells from progenitors committed to this lineage occurs before birth in the fetal liver and after birth in the bone marrow, with the final steps being completed in the spleen. Fetal liver–derived stem cells give rise mainly to a type of B cell called a B-1 cell, whereas bone marrow–derived HSCs give rise to the majority of circulating B cells (follicular B cells) and a subset of B cells called marginal zone B cells. Precursors of T lymphocytes emerge from the fetal liver before birth and from the bone marrow later in life and circulate to the thymus, where they complete their maturation. T cells that express γδ T cell receptors (TCRs) arise from fetal liver HSCs, and the majority of T cells, which express αβ TCRs, develop from bone marrow–derived HSCs. In general, the B and T cells that are generated early in fetal life have less diverse antigen receptors. Despite their different anatomic locations, the early maturation events of both B and T lymphocytes are fundamentally similar.

Commitment of common lymphoid progenitors to the B or T cell lineage depends on transcriptional regulators that drive development toward either B cells or T cells. Key events in the commitment of precursor cells to the B cell or T cell lineage are expression of the proteins involved in antigen receptor gene rearrangements, described later in the chapter, and the generation of accessibility, at the level of chromatin, of particular antigen receptor gene loci to these proteins. In the case of developing B cells, the immunoglobulin (Ig) heavy chain locus, initially in a closed chromatin configuration, is opened so that it becomes accessible to the proteins that will mediate Ig gene rearrangement and expression. In developing αβ T cells, the TCR β gene locus is made accessible first. These changes in the chromatin accessibility of antigen receptor loci during development are initiated by sets of lineage-specific transcription factors.

Numerous transcription factors are involved in the maturation of T and B cells (see Fig. 8.2). Notch1 and GATA3 commit developing lymphocytes to the T cell lineage. The Notch family of proteins are cell surface molecules that are proteolytically cleaved when they interact with specific ligands on neighboring cells (see Fig. 7.2). The cleaved intracellular portions of Notch proteins migrate to the nucleus and modulate the expression of specific target genes. Notch1 is activated in lymphoid progenitor cells, and together with GATA3 it induces expression of a number of genes that are required for the further development of αβ T cells. Some of these genes encode components of the pre-T cell receptor (pre-TCR) and the RAG1 and RAG2 proteins, which are required for V(D)J recombination, described later. The EBF, E2A, and PAX5 transcription factors induce the expression of genes required for B cell development. These include genes encoding the RAG1 and RAG2 proteins, components of the pre-B cell receptor (pre-BCR), and proteins that contribute to signaling through the pre-BCR and the BCR. The role of these proteins in T and B cell development will be considered later in this chapter.

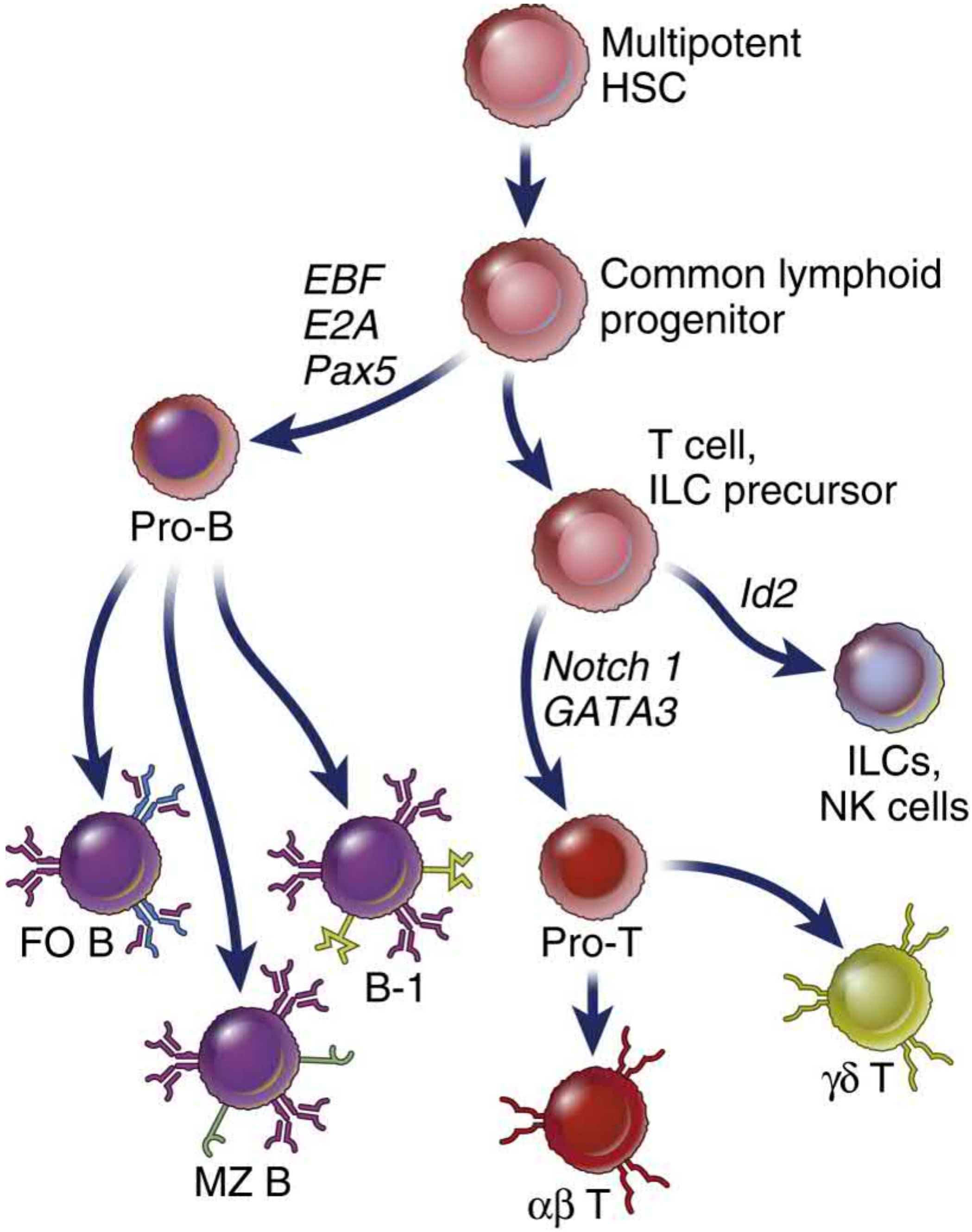

Multipotent HSC
Common lymphoid progenitor
EBF
E2A
Pax5
Pro-B
T cell, ILC precursor
Id2
Notch 1
GATA3
ILCs, NK cells
FO B
B-1
MZ B
Pro-T
αβ T
γδ T

FIGURE 8.2 Multipotent stem cells give rise to distinct B and T lineages.Hematopoietic stem cells *(HSCs)* give rise to distinct progenitors for various types of blood cells. One of these progenitor populations (shown here) is called a common lymphoid progenitor (CLP). CLPs give rise to B and T cells and also contribute to natural killer *(NK)* cells, innate lymphoid cells *(ILCs),* and some

dendritic cells (not depicted here). Pro-B cells can eventually differentiate into follicular *(FO)* B cells, marginal zone *(MZ)* B cells, and B-1 cells. Pro-T cells may commit to either the αβ or γδ T cell lineages. Commitment to different lineages is driven by various transcription factors, indicated in italics.

During B and T cell development, committed progenitor cells proliferate first in response to cytokines and later in response to signals generated by a pre-antigen receptor that select cells that have successfully rearranged the first set of antigen receptor genes. Proliferation ensures that a large enough pool of progenitor cells will be generated to eventually produce a highly diverse repertoire of mature, antigen-specific lymphocytes. In mice, widely used for basic research of lymphocyte development, the cytokine interleukin-7 (IL-7) drives proliferation of early T and B cell progenitors; in humans, IL-7 is required for the proliferation of T cell progenitors but not of progenitors in the B lineage. The factors that drive the proliferation of human progenitor B cells remain to be identified. IL-7 is produced by stromal cells in the bone marrow and by epithelial and other cells in the thymus. Mice with targeted mutations in the gene encoding either IL-7 or the IL-7 receptor show defective maturation of lymphocyte precursors beyond the earliest stages and, as a result, profound deficiencies in mature T and B cells. Mutations in the human gene encoding the common γ chain, a protein that is shared by the receptors for several cytokines, including IL-2, IL-7, and IL-15, give rise to an immunodeficiency disorder called **X-linked severe combined immunodeficiency disease (X-SCID)** (see Chapter 21). This disease is characterized by a block in T cell and NK cell development, but normal B cell development, because IL-7 is required for T cell development in humans and IL-15 for NK cells.

The greatest proliferative expansion of lymphocyte precursors occurs after successful rearrangement of the genes encoding one of the two chains of the T or B cell antigen receptor, producing a pre-antigen receptor (described later). Signals generated by pre-antigen receptors are responsible for far greater proliferation of developing lymphocytes (which have successfully rearranged the Ig heavy chain gene or the TCR β chain gene, as the case may be) than the earlier proliferation driven by cytokines such as IL-7.

Role of Epigenetic Changes and MicroRNAs in Lymphocyte Development

Many nuclear events in lymphocyte development are regulated by epigenetic mechanisms. Epigenetics refers to the control of gene expression and phenotypes by mechanisms other than changes in the coding sequences themselves. In developing lymphocytes, epigenetic mechanisms also control antigen receptor gene rearrangement events. DNA exists in chromosomes tightly bound to histones and nonhistone proteins, forming what is known as chromatin. DNA in chromatin is wound around a protein core of histone octamers, forming structures called nucleosomes, which may be either well separated from other nucleosomes or densely packed. Chromatin may therefore exist as relatively loosely packed structures, called euchromatin, wherein genes can be accessed by transcription factors and are transcribed, or as tightly packed heterochromatin in which genes are maintained in a silenced state. The structural organization of portions of chromosomes varies in different cells, making certain genes available for transcription factors to bind to, while these very same genes may be unavailable to transcription factors in other cells. Epigenetic mechanisms regulate the accessibility and activity of genes by inducing changes in promoter and enhancer regions of genes. These changes include: the methylation of DNA on certain cytosine residues that generally silences genes; post-translational modifications of the histone tails of nucleosomes (e.g., acetylation, methylation, and ubiquitination) that may render genes either active or inactive depending on the histone modified and the nature of the modification; active remodeling of chromatin by protein machines called remodeling complexes that can also either enhance or suppress gene expression; and the silencing of gene expression by noncoding RNAs.

Some critical components of lymphocyte development are regulated by epigenetic mechanisms.

- Histone modifications in antigen receptor gene loci are required for recruitment of proteins that mediate gene recombination to form functional antigen receptor genes. This process is discussed later in the chapter.
- Commitment of developing T cells to the CD4 or CD8 lineage depends on epigenetic mechanisms that silence the expression of the CD4 gene in $CD8^+$ T cells. Silencing involves chromatin modifications that place the CD4 gene into an inaccessible heterochromatin state.
- In Chapter 7, we discussed microRNAs (miRNAs) in the context of T cell activation. They contribute in significant ways to modulating gene and protein expression during development as well. As mentioned in Chapter 7, Dicer is a key enzyme in miRNA generation. Deletion of Dicer in the T lineage results in a preferential loss of regulatory T cells and the consequent development of an autoimmune phenotype similar to that seen in the absence of FOXP3 (discussed in Chapters 15 and 21). The loss of Dicer in the B lineage results in a block at the pro-B to pre-B cell transition (discussed in more detail later), primarily due to enhanced apoptosis of pre-B cells. Gene ablation studies have revealed that many specific miRNAs are involved in lymphocyte development.

Antigen Receptor Gene Rearrangement and Expression

The rearrangement of antigen receptor genes is an essential event in lymphocyte development, and this process is responsible for the

generation of a diverse adaptive immune repertoire. As we discussed in previous chapters, each clone of B or T lymphocytes produces an antigen receptor with a unique antigen-binding structure. In any individual, there may be 10^7 to 10^9 different B and T lymphocyte clones, each with a unique receptor. The ability of each individual to generate these large and diverse lymphocyte repertoires has evolved in such a way that a fairly small number of genes can give rise to a vast number of distinct Ig and TCR molecules, each capable of binding to a different antigen. Functional antigen receptor genes are produced in immature B cells in the bone marrow and in immature T cells in the thymus by a process of gene rearrangement. In this process, segments of antigen receptor genes are randomly recombined and nucleotide sequence variations are introduced at the joints, resulting in the production of a large number of variable region–encoding exons. The DNA rearrangement events that lead to the production of antigen receptors are not dependent on or influenced by the presence of antigens. In other words, as the clonal selection hypothesis had proposed, diverse antigen receptors are generated and expressed before encounter with antigens (see Fig. 1.7). We will discuss the molecular details of antigen receptor gene rearrangement later in this chapter.

Selection Processes That Shape the B and T Lymphocyte Repertoires

The process of lymphocyte development contains numerous steps, called checkpoints, at which the developing cells are tested and continue to mature only if a preceding step in the process has been successfully completed. One of these developmental checkpoints is based on the successful production of one of the polypeptide chains of the two-chain antigen receptor protein, and a second checkpoint requires the second chain and thus assembly of a complete receptor. The requirement for traversing these developmental checkpoints is a quality control mechanism that ensures that only lymphocytes that produce complete antigen receptors and are therefore likely to be functional are selected to mature. Additional selection processes operate after antigen receptors are expressed and serve to eliminate potentially harmful, self-reactive lymphocytes and to commit developing cells to particular lineages. (Note that the term checkpoints is also used to describe very different phenomena in the context of peripheral immune activation and cancer immunotherapy [see Chapter 18]). We will next summarize the general principles of these events.

Pre-antigen receptors and antigen receptors deliver signals to developing lymphocytes that are required for the survival of these cells and for their proliferation and continued maturation (Fig. 8.3) **.** Pre-antigen receptors, called pre-BCRs in B cells and pre-TCRs in T cells, are signaling structures expressed during B and T cell development that contain only one of the two polypeptide chains present in a mature antigen receptor. Cells of the B lymphocyte lineage that successfully rearrange their Ig heavy chain genes express the μ heavy chain protein and assemble a pre-BCR. In an analogous fashion, developing T cells that make a successful TCR β chain gene rearrangement synthesize the TCR β chain protein and assemble a pre-TCR. The assembled pre-BCR and pre-TCR form complexes with proteins that generate signals for survival, proliferation, and the phenomenon of allelic exclusion (discussed later), and for the further development of B and T cells. Because of the random addition of nucleotides at junctions between segments of antigen receptor genes that are joined together during lymphocyte development and the triplet base pair code for determining amino acids, only about one in three antigen receptor gene rearrangements is in frame and, therefore, capable of generating a proper full-length protein. Such a successful rearrangement is sometimes called a productive rearrangement. If cells make out-of-frame or nonproductive gene rearrangements at the Ig μ or TCR β chain loci, the pre-antigen receptors are not expressed, the cells do not receive necessary survival signals, and they undergo programmed cell death. Thus, expression of the pre-antigen receptor is the first checkpoint during lymphocyte development.

In the next step of maturation, developing B and T cells express complete antigen receptors and the cells are selected for survival. Lymphocytes that have successfully navigated the pre–antigen receptor checkpoint go on to rearrange and express genes encoding the second chain of the BCR or TCR and express the complete antigen receptor while they are still immature. At this immature stage, cells that express useful antigen receptors may be preserved, and potentially harmful cells that strongly recognize self antigens may be eliminated or induced to alter their antigen receptors (see Fig. 8.3).

A process called **positive selection** facilitates the survival of potentially useful lymphocytes. In the T cell lineage, positive selection ensures the maturation of T cells whose receptors recognize self major histocompatibility complex (MHC) molecules. Also, the expression of the coreceptor on a T cell (CD8 or CD4) is matched to the recognition of the appropriate type of MHC molecule (class I MHC or class II MHC, respectively). Mature T cells whose precursors were positively selected by self MHC molecules in the thymus are able to recognize foreign peptide antigens displayed by the same self MHC molecules on antigen-presenting cells (APCs) in peripheral tissues. In the B cell lineage, positive selection preserves receptor-expressing cells and is coupled to the generation of different B cell subsets.

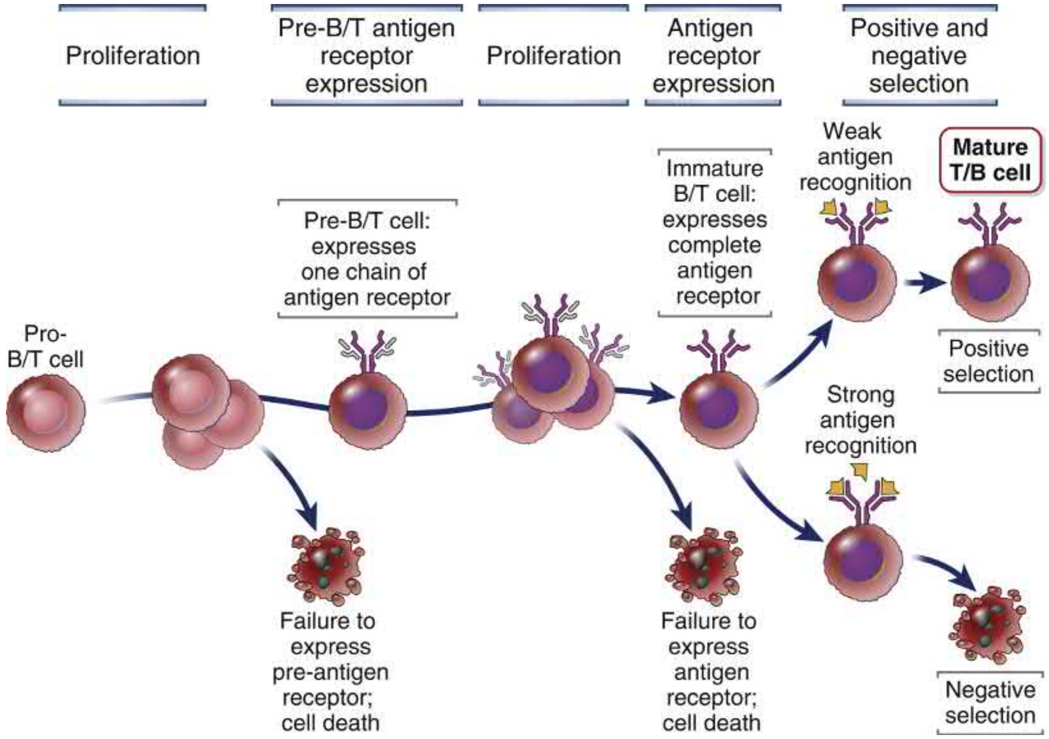

Proliferation
Pre-B/T antigen receptor expression
Proliferation
Antigen receptor expression
Positive and negative selection
Pro-B/T cell
Pre-B/T cell: expresses one chain of antigen receptor
Immature B/T cell: expresses complete antigen receptor
Weak antigen recognition
Mature T/B cell
Positive selection
Strong antigen recognition
Failure to express pre-antigen receptor; cell death
Failure to express antigen receptor; cell death
Negative selection

FIGURE 8.3 Checkpoints in lymphocyte maturation.During development, the lymphocytes that express receptors required for continued proliferation and maturation are selected to survive, and cells that do not express functional receptors die by apoptosis. Positive and negative selection further preserve cells with useful specificities. The presence of multiple checkpoints ensures that only cells with useful receptors complete their maturation.

Negative selection is the process that eliminates or alters developing lymphocytes whose antigen receptors bind strongly to self antigens present in the generative lymphoid organs. Developing B and T cells are susceptible to negative selection during a short period after antigen receptors are first expressed. Developing T cells with a high affinity for self antigens are eliminated by apoptosis, a phenomenon known as **clonal deletion.** Strongly self-reactive immature B cells may be induced to make further Ig gene rearrangements and thus avoid self reactivity. This phenomenon is called **receptor editing.** If editing fails, the self-reactive B cells die, which is also called clonal deletion. Negative selection of immature lymphocytes is an important mechanism for maintaining tolerance to many self antigens; this is also called **central tolerance** because it develops in the central (generative) lymphoid organs (see Chapter 15).

With this introduction, we will proceed to a more detailed discussion of lymphocyte maturation, starting with the key event in the process, the rearrangement and expression of antigen receptor genes.

Rearrangement of Antigen Receptor Genes in B and T Lymphocytes

The genes that encode diverse antigen receptors of individual B and T lymphocytes are generated by the recombination of different variable (V) region gene segments with diversity (D) and joining (J) gene segments. This specialized process of site-specific gene rearrangement is called **V(D)J recombination.** Elucidation of the mechanisms of antigen receptor gene rearrangement, and therefore of the underlying basis for the generation of lymphocyte diversity, represents one of the landmark achievements of modern immunology.

The first insights into how millions of different antigen receptors could be generated from a limited amount of coding DNA in the genome came from analyses of the amino acid sequences of Ig molecules. These analyses showed that the polypeptide chains of many different antibodies of the same isotype shared identical sequences at their C-terminal ends (corresponding to the constant domains of antibody heavy and light chains) but differed considerably in the sequences at their N-terminal ends that correspond to the variable domains of antibodies (see Chapter 5). Contrary to one of the central tenets of molecular genetics, enunciated as the one gene–one polypeptide hypothesis, immunologists postulated in 1965 that each antibody chain is actually encoded by at least two genes, one variable and the other constant, and that the two are physically combined at the level of DNA or of messenger RNA (mRNA) to eventually give rise to functional Ig proteins. Formal proof of this hypothesis came more than a decade later when Susumu Tonegawa demonstrated that the structure of Ig genes in the cells of an antibody-producing tumor, called a myeloma or plasmacytoma, is different from that in embryonic tissues or in nonlymphoid tissues not committed to Ig production. These differences arise because DNA segments encoding Ig heavy and light chains are separated within the inherited (or germline) loci and are brought together and joined only in developing B cells but not in other tissues or cell types. Similar rearrangements were found to occur during T cell development in the loci encoding the polypeptide chains of TCRs. Antigen receptor gene rearrangement is best understood by first describing the inherited unrearranged (germline) organization of Ig and TCR genes and then describing their rearrangement during lymphocyte maturation.

Germline Organization of Immunoglobulin and T Cell Receptor Genes

Germline Ig and TCR genes are composed of multiple DNA segments that are spatially separate in all cells and are combined in developing lymphocytes. We will first describe the Ig loci and then the TCR loci.

Organization of Immunoglobulin Gene Loci

Three separate loci encode, respectively, all of the Ig heavy chains, the Ig κ light chain, and the Ig λ light chain. Each locus is on a different chromosome. The organization of human Ig genes is illustrated in Fig. 8.4. Ig genes are organized in essentially the same way in all mammals, although their chromosomal locations and the number and order of different gene segments in each locus may vary.

At the 5′ end of each Ig locus, there is a cluster of variable (V) gene segments, each about 300 base pairs long. The numbers of functional V gene segments vary considerably among the different Ig loci and among different species. For example, in humans there are about 35 functional V gene segments in the κ light chain locus, about 30 in the λ locus, and about 45 in the heavy chain locus; whereas in mice, there are about 30 functional V gene segments in the κ locus, only two in the λ light chain locus, and about 250 in the heavy chain locus. In both species V gene segments for each locus are spaced over large stretches of DNA, up to 2000 kilobases long. Located 5′ of each V gene segment is a leader exon that encodes the 20 to 30 N-terminal residues of the translated protein. These residues are moderately hydrophobic and make up the leader (or signal) peptide. Signal sequences are found in all newly synthesized secreted and transmembrane proteins and are involved in guiding nascent polypeptides being translated on ribosomes to bind to a cytosolic complex that docks these specific ribosomes onto the endoplasmic reticulum membrane to allow protein translocation into the lumen of the endoplasmic reticulum. Here the signal sequences are rapidly cleaved, and they are not present in the mature proteins. Upstream of each leader exon is a V gene segment promoter at which transcription can be initiated, but as discussed later, this occurs most efficiently after rearrangement.

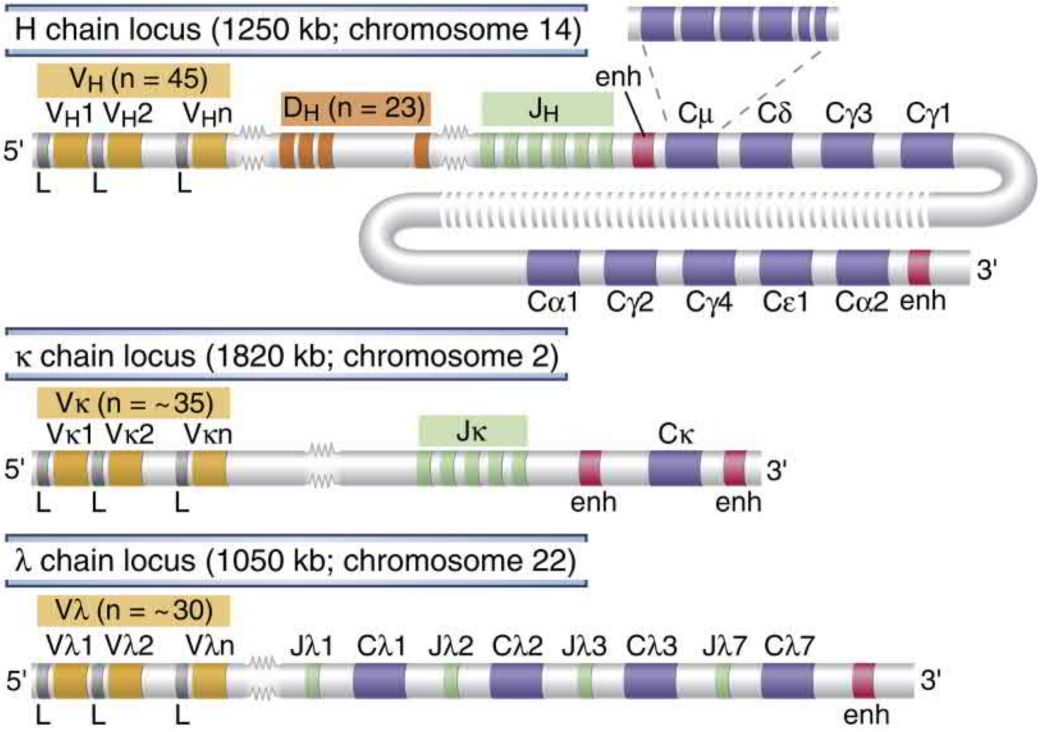

H chain locus (1250 kb; chromosome 14)
VH (n = 45)
VH1 VH2 VHn
DH (n = 23)
JH
enh
Cμ
Cδ
Cγ3
Cγ1
5'
L L L
3'
Cα1 Cγ2 Cγ4 Cε1 Cα2 enh
κ chain locus (1820 kb; chromosome 2)
Vκ (n = ~35)
Vκ1 Vκ2 Vκn
Jκ
Cκ
5'
3'
L L L
enh
enh
λ chain locus (1050 kb; chromosome 22)
Vλ (n = ~30)
Vλ1 Vλ2 Vλn
Jλ1 Cλ1 Jλ2 Cλ2 Jλ3 Cλ3 Jλ7 Cλ7
5'
3'
L L L
enh

FIGURE 8.4 Germline organization of human immunoglobulin gene loci. The human heavy chain, κ light chain, and λ light chain genes are shown. Only functional gene segments are shown; pseudogenes have been omitted for simplicity. Exons and introns are not drawn to scale. Each C_H gene is shown as a single box but is composed of several exons, as illustrated for Cμ. Gene segments are indicated as follows: *C,* constant; *D,* diversity; *enh,* enhancer; *J,* joining; *L,* leader (often called signal sequence); *V,* variable. In this and in subsequent figures, the tubular structures depict double-stranded segments of chromosomes, with the 5′ and 3′ ends referring to the coding strands.

At varying distances 3′ of the V gene segments are several joining (J) segments that are typically 30 to 50 base pairs long and are separated by noncoding sequences. Between the V and J segments in the Ig heavy chain (IgH) locus, there are additional segments known as diversity (D) segments. D segments are not found in Ig light chain loci. Like V gene segments, the numbers of D and J segments vary in different Ig loci and different species.

The constant (C) region genes are located 3′ of the J segments. Each Ig locus has a distinct arrangement and number of C region genes. In humans, the Ig κ light chain locus has a single C gene (Cκ), and the λ light chain locus has four functional C genes (Cλ). The Ig heavy chain locus has nine C genes (C_H), arranged in a tandem array, that encode the C regions of the nine different Ig isotypes and subtypes (see Chapter 5). The Cκ and Cλ genes are each composed of a single exon that encodes the entire C domain of the light chains. In contrast, each C_H gene is composed of five or six exons. Three or four exons (each similar in size to a V gene segment) each encode a C_H domain of the Ig heavy chain, and two smaller exons code for the carboxy-terminal ends of the membrane form of each Ig heavy chain, including the transmembrane and cytoplasmic domains of the heavy chains (Fig. 8.5A).

The V, J, and D (if present) gene segments are brought together to create the coding sequence for the variable domains of antibody chains (see Fig. 8.5A). In an Ig light chain protein (κ or λ), the V domain is encoded by the rearranged V and J gene segments; in the Ig heavy chain protein, the V domain is encoded by the recombined V, D, and J gene segments. In the case of Ig heavy chain V domains, the non-germline junctional residues between the rearranged V and D segments and the D and J segments, as well as the germline sequences of the D and J segments themselves, make up the third hypervariable region, also known as complementarity-determining region 3 (CDR3) (see Chapter 5). The junctional sequences between the rearranged V and J segments as well as the J segment itself make up the third hypervariable region of Ig light chains. CDR1 and CDR2 are encoded in the V gene segment only.

A complete Ig light chain or heavy chain protein contains a V domain encoded by a rearranged VJ or VDJ exon, fused to a C domain or domains. The apposition of Ig V and C domains does not occur at the level of DNA rearrangement but by RNA-splicing of the rearranged Ig gene transcript.

Noncoding sequences in the Ig loci play important roles in recombination and gene expression. As we will see later, sequences that dictate recombination of different gene segments are found adjacent to each coding segment in Ig genes. Also present are V gene promoters and other *cis*-acting regulatory elements, such as locus control regions, enhancers, and silencers, which regulate gene expression at the level of transcription.

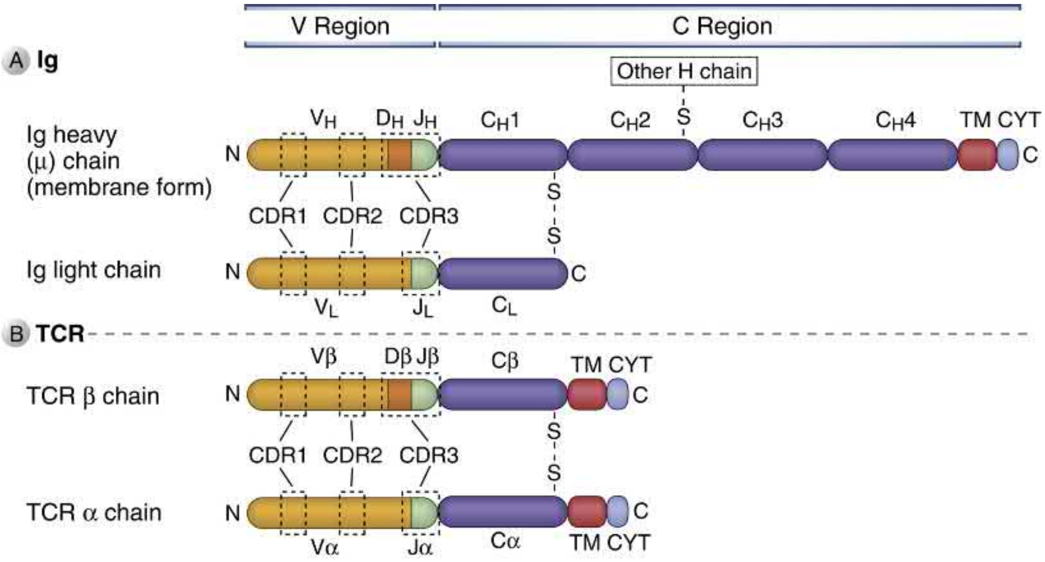

V Region
C Region
A Ig
Other H chain
Ig heavy (μ) chain (membrane form)
N
VH
DH
JH
CH1
CH2
S
CH3
CH4
TM CYT
C
CDR1
CDR2
CDR3
S
S
Ig light chain
N
C
VL
JL
CL
B TCR
Vβ
Dβ Jβ
Cβ
TM CYT
TCR β chain
N
C
CDR1
CDR2
CDR3
S
S
TCR α chain
N
C
Vα
Jα
Cα
TM CYT

FIGURE 8.5 Domains of immunoglobulin and T cell receptor proteins. The domains of immunoglobulin (Ig) heavy and light chains are shown in **A,** and the domains of T cell receptor (TCR) α and β chains are shown in **B.** The relationships between the Ig and TCR gene segments and the domain structure of the antigen receptor polypeptide chains are indicated. The V and C regions of each polypeptide are encoded by different gene segments. The locations of intrachain and interchain disulfide bonds (S-S) are approximate. Areas in the *dashed boxes* are the hypervariable (complementarity-determining) regions. In the Ig μ chain and the TCR α and β chains, transmembrane *(TM)* and cytoplasmic *(CYT)* domains are encoded by separate exons. *C,* Carboxy termini; *N,* amino termini.

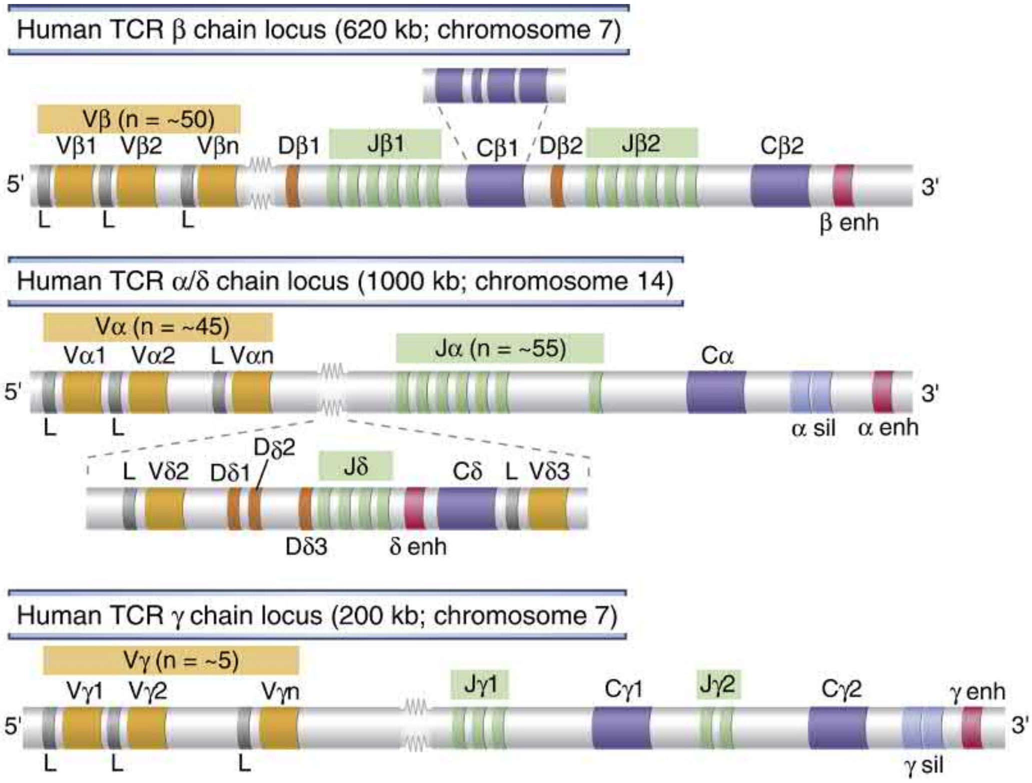

Human TCR β chain locus (620 kb; chromosome 7)
Vβ (n = ~50)
Vβ1
Vβ2
Vβn
Dβ1
Jβ1
Cβ1
Dβ2
Jβ2
Cβ2
5'
3'
L
L
L
β enh
Human TCR α/δ chain locus (1000 kb; chromosome 14)
Vα (n = ~45)
Vα1
Vα2
L Vαn
Jα (n = ~55)
Cα
5'
3'
L
L
α sil
α enh
Dδ2
L Vδ2
Dδ1
Jδ
Cδ
L Vδ3
Dδ3
δ enh
Human TCR γ chain locus (200 kb; chromosome 7)
Vγ (n = ~5)
Vγ1
Vγ2
Vγn
Jγ1
Cγ1
Jγ2
Cγ2
γ enh
5'
3'
L
L
L
γ sil

FIGURE 8.6 Germline organization of human T cell receptor gene loci. The human T cell receptor *(TCR)* β, α, γ, and δ chain genes are shown, as indicated. Exons and introns are not drawn to scale, and nonfunctional pseudogenes are not shown. Each C gene is shown as a single box but is composed of several exons, as illustrated for Cβ1. Gene segments are indicated as follows: *C,* constant; *D,* diversity; *enh,* enhancer; *J,* joining; *L,* leader (usually called signal sequence); *sil,* silencer (sequences that regulate *TCR* gene transcription); *V,* variable.

Organization of T Cell Receptor Gene Loci

Each germline TCR locus is arranged in a very similar way to the Ig loci described earlier, with a 5′ cluster of several V gene segments, followed by D segments (in the β and δ loci only), followed by a cluster of J segments, all upstream of C region genes (Fig. 8.6). In the human β locus there are about 50 V, 2 D, and 12 J gene segments, and in the α locus there are 45 V and 50 J segments. The γ and δ loci overall have fewer gene segments than the α and β loci, with a total of only 7 V genes. Upstream of each TCR V gene segment is an exon that encodes a leader peptide, and upstream of each leader exon is a promoter for each V gene segment. In the TCR β and δ proteins, the V domain is encoded by the V, D, and J gene segments, and in the TCR α and γ proteins, the V domain is encoded by the V and J gene segments. In all these V domains, CDR1 and CDR2 are encoded by germline sequences within V gene segments. CDR3 in each TCR β and TCR δ chain is encoded by a D and a J segment as well as non-germline junctional sequences that are added between the V, D, and J segments. CDR3 in each α and γ chain is encoded by non-germline junctional sequences between the V and J segment and by the J segment itself. There are two C genes in each of the human TCR β and TCR γ loci, but only one is used in any T cell clone, and each of the two has its own associated 5′ cluster of J segments. There is only one C gene in each of the α and δ loci. Each TCR C region gene is composed of four exons encoding the extracellular C region Ig domain, a short hinge region, the transmembrane segment, and the cytoplasmic tail.

The relationship of the TCR gene segments and the corresponding portions of TCR proteins that they encode is shown in Fig. 8.5B.

V(D)J Recombination

The germline organization of Ig and TCR loci described in the preceding section exists in all cell types in the body. The germline genes cannot be transcribed into mRNAs that encode functional antigen receptor proteins. Functional antigen receptor genes are created only in developing B and T lymphocytes after DNA rearrangement brings randomly chosen V, D (if present), and J gene segments into contiguity.

The process of V(D)J recombination at any Ig or TCR locus involves the rearrangement of one V gene segment, one D segment

(only in Ig heavy chain and TCR β and δ chain loci), and one J segment in each lymphocyte to form a single V(D)J exon that will code for the variable region of an antigen receptor protein (Fig. 8.7) **.** In the Ig light chain and TCR α and γ loci, which lack D segments, a single rearrangement event joins a randomly selected V gene segment to a randomly selected J segment. The Ig H and TCR β and δ loci contain D segments, and at these loci two sequential rearrangement events are needed, first joining a D to a J and then a V segment to the fused DJ segment, with each segment type randomly selected from the inherited group of V, D, or J segments. Each rearrangement event involves a number of steps. First, the chromatin is opened in specific regions of the chromosome to make antigen receptor gene segments accessible to the enzymes that mediate recombination. Next, two selected gene segments are brought next to one another across a considerable chromosomal distance. Double-stranded breaks are then introduced at the coding ends of these two segments, nucleotides are added or removed at the broken ends, and finally the processed ends are ligated to produce antigen receptor genes that can be efficiently transcribed. The C regions lie downstream of the rearranged V(D)J exon separated by the germline J-C intron. This rearranged gene is transcribed to form a primary (nuclear) RNA transcript. Subsequent RNA splicing brings together the leader exon, the V(D)J exon, and the C region exons, forming an mRNA that can be translated to produce one of the chains of the antigen receptor. The use of different combinations of V, D, and J gene segments and the addition and removal of nucleotides at the junctions contribute to the tremendous diversity of antigen receptors, as we will discuss in more detail later. Also, because the gene segment combinations and the junctions between them are different in each developing B or T lymphocyte, each cell and its clonal progeny produce a distinct antigen receptor.

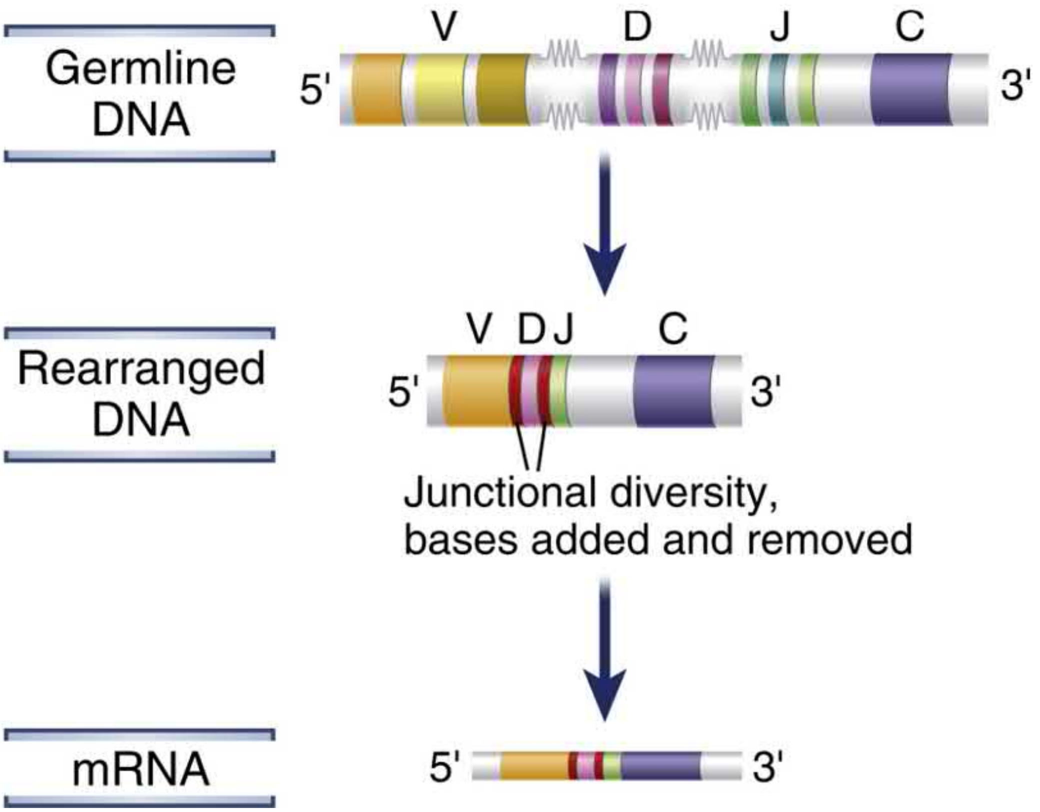


FIGURE 8.7 An overview of V(D)J recombination.Only a small number of V, D, and J gene segments are shown for simplicity.

Recognition Signals That Drive V(D)J Recombination

Lymphocyte-specific proteins that mediate V(D)J recombination recognize DNA sequences called recombination signal sequences (RSSs) that are located 3′ of each V gene segment, 5′ of each J segment, and flanking each side of every D segment (Fig. 8.8A) **.** The RSSs consist of a conserved stretch of 7 nucleotides, called the heptamer, usually CACAGTG, located adjacent to the coding sequence, followed by a spacer of either 12 or 23 non-conserved nucleotides, followed by a conserved AT-rich stretch of 9 nucleotides, called the nonamer. Specific residues in the heptamer and nonamer contribute to the binding of the recombinase enzyme that mediates joining that will be described in the next section. The 12- and 23-nucleotide spacers roughly correspond to one or two turns of a DNA helix, respectively, and they ensure that two distinct RSSs, each adjacent to a different type of coding gene segment, are brought close to one another for recombination, as discussed in the following section.

During V(D)J recombination, double-strand breaks are generated between the heptamer of the RSS and the adjacent V, D, or J coding sequence. In Ig light chain V-to-J recombination, for example, breaks will be made 3′ of a V segment and 5′ of a J segment. The intervening DNA, containing signal ends (the ends that contain the heptamer and the rest of the RSS), is removed in the form of a circle, and the V and J coding ends are joined (Fig. 8.8B). In some V gene segments, especially in the Ig κ locus, the RSSs are 3′ of a Vκ and 3′ of Jκ, and therefore do not face each other. In these cases, the intervening DNA is inverted and the V and J segments are properly aligned; the fused RSSs are not deleted but retained in the chromosome (Fig. 8.8C). Most Ig and TCR gene rearrangements occur by deletion; inversion is the basis of up to 50% of rearrangements in the Ig κ locus. Recombination occurs between two segments only if one of the segments is flanked by a 12-nucleotide spacer and the other is flanked by a 23-nucleotide spacer. This is called the 12/23 rule. Thus, the location of flanking RSSs ensures that the appropriate gene segments will recombine. For example, in the Ig heavy chain locus, the RSSs flanking both V and J segments have 23-nucleotide spacers (two turns of the DNA helix) and therefore cannot join directly; D-to-J recombination occurs first, followed by V-to-DJ recombination. This is possible because the D segments are flanked on both sides by 12-nucleotide spacers, allowing D-J and then V-DJ joining. The RSSs described here are unique to Ig and TCR genes. Therefore, V(D)J recombination can occur in antigen receptor genes but not in other genes.

One of the consequences of V(D)J recombination is that the process brings promoters located immediately 5′ of V genes close to downstream enhancers that are located in the introns between J and C segments and also 3′ of the C region genes (Fig. 8.9). These enhancers maximize the transcriptional activity of the V gene promoters and are thus important for high-level transcription of rearranged V genes in lymphocytes. Because Ig and TCR genes are sites for multiple DNA recombination events in B and T cells, and because these sites become transcriptionally active after recombination, genes from other loci can be abnormally translocated to these loci and, as a result, may be aberrantly transcribed. In tumors of B and T lymphocytes, oncogenes are often translocated to Ig or TCR gene loci. Such chromosomal translocations are frequently accompanied by enhanced transcription of the oncogenes and are a major mechanism leading to the development of lymphoid tumors.

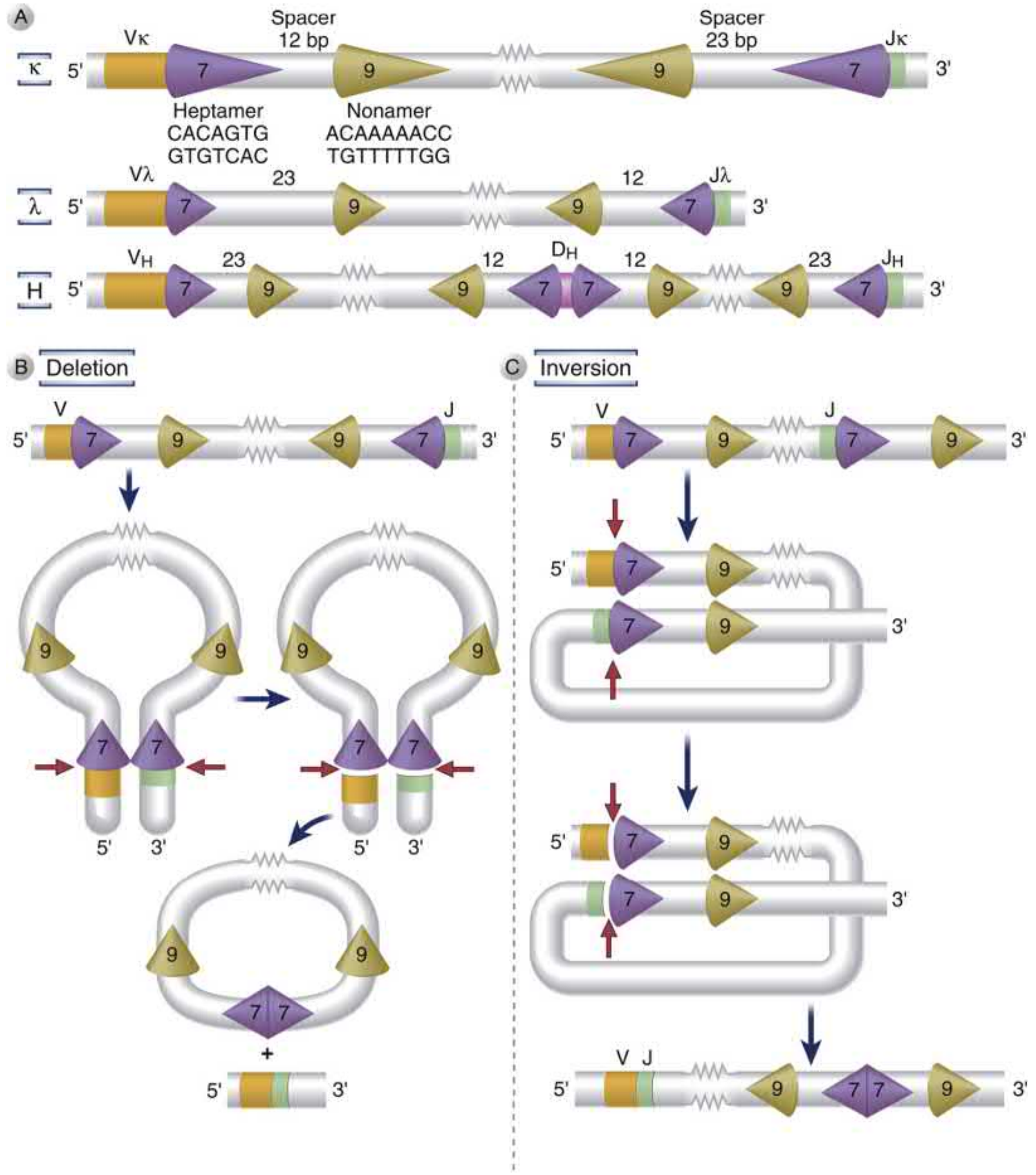

A
κ
5'
Vκ
7
Spacer
12 bp
9
9
Spacer
23 bp
7
Jκ
3'
Heptamer
CACAGTG
GTGTCAC
Nonamer
ACAAAAACC
TGTTTTTGG
λ
5'
Vλ
7
23
9
9
12
7
Jλ
3'
H
5'
VH
7
23
9
9
12
DH
7
7
12
9
9
23
7
JH
3'
B
Deletion
5'
V
7
9
9
7
J
3'
9
9
7
7
5'
3'
9
9
7
7
5'
3'
9
9
7 7
+
5'
3'
C
Inversion
5'
V
7
9
J
7
9
3'
5'
7
9
7
9
3'
5'
7
9
7
9
3'
5'
V J
9
7 7
9
3'

FIGURE 8.8 V(D)J recombination. The DNA sequences and mechanisms involved in recombination in the immunoglobulin (Ig) gene loci are depicted. The same sequences and mechanisms apply to recombinations in the T cell receptor (TCR) loci. **A,** Conserved heptamer (7 bp) and nonamer (9 bp) sequences, separated by 12- or 23-bp spacers, are located adjacent to V and J segments (for κ and λ loci) or to V, D, and J segments (in the H chain locus). The V(D)J

recombinase recognizes these recombination signal sequences and brings the exons together. **B** and **C,** Recombination of V and J exons may occur by deletion of intervening DNA and ligation of the V and J segments **(B)** or, if the RSS is 3′ of a J segment, by inversion of the DNA followed by ligation of adjacent gene segments **(C)**. *Red arrows* indicate the sites where germline sequences are cleaved before their ligation to other Ig or TCR gene segments.

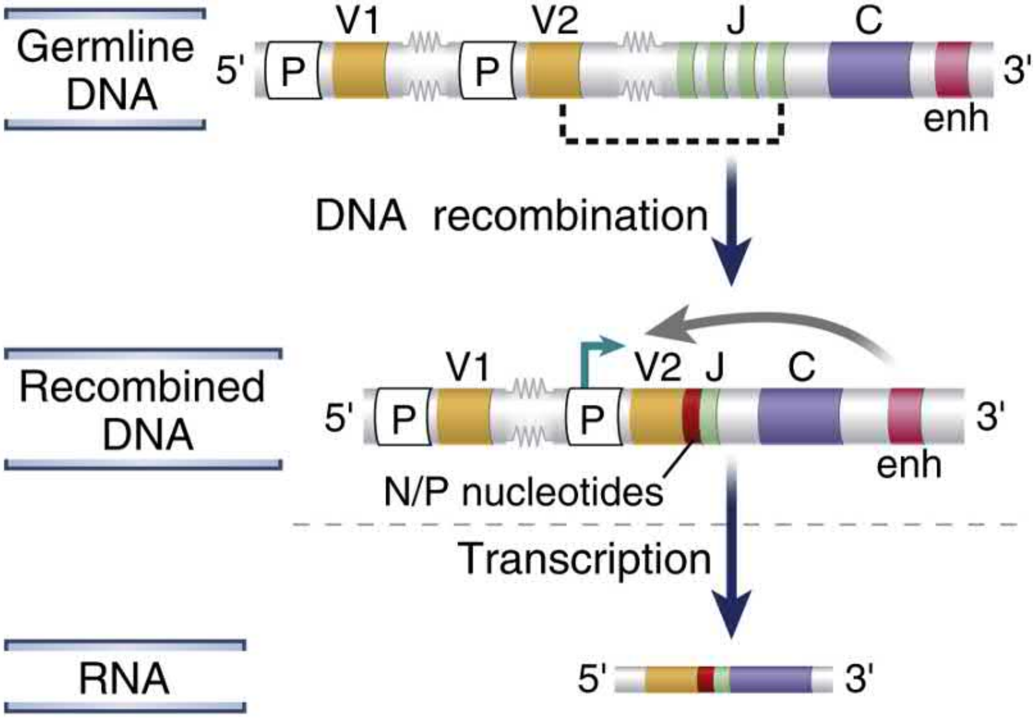

Germline DNA
5'
P
V1
P
V2
J
C
3'
enh
DNA recombination
Recombined DNA
5'
P
V1
P
V2
J
C
3'
enh
N/P nucleotides
Transcription
RNA
5'
3'

FIGURE 8.9 Transcriptional regulation of immunoglobulin genes. V(D)J recombination brings inactive promoter sequences (shown as *P*) close to the enhancer *(enh).* The enhancer promotes transcription of the rearranged V gene (V2, whose active promoter is indicated by a *bold blue arrow*). Several receptor genes have an enhancer in the J-C intron and another 3′ of the C region. Only the 3′ enhancer is depicted here.

Mechanism of V(D)J Recombination

Rearrangement of Ig and TCR genes represents a special kind of nonhomologous DNA recombination event that is mediated by the coordinated activities of several enzymes. Some of these enzymes are found only in developing lymphocytes, whereas others are ubiquitous DNA double-stranded break repair (DSBR) enzymes. Although the mechanism of V(D)J recombination is fairly well understood and will be described here, how exactly specific loci are made accessible to the machinery involved in recombination remains to be determined. It is likely that the accessibility of the Ig and TCR loci to the enzymes that mediate recombination is regulated in developing B and T cells by several mechanisms, including epigenetic alterations in chromatin structure and basal transcriptional activity in the gene loci. There are two main steps involved in achieving accessibility. First, only RSSs that are located in open euchromatin in a specific cell type will be exposed to recombination enzymes. For example, the IgH, Igκ, and Igλ loci will be exposed in a B cell but not in a developing T cell. Secondly, within this open euchromatin state, gene segments that are actually undergoing recombination acquire additional histone marks, such as the hypermethylation of lysine 4 on histone 3 (H3K4). This modification specifically facilitates recruitment of enzymes, as discussed shortly.

The process of V(D)J recombination can be divided into four distinct sequential events (Fig. 8.10):

1. ***Synapsis.*** Synapsis refers to the process by which two distant selected coding segments and their adjacent RSSs that have acquired specific histone marks are brought together by a chromosomal looping event and held in position for subsequent cleavage, processing, and joining.
2. ***Cleavage.*** A lymphocyte-specific enzyme called the **V(D)J recombinase** creates double-stranded breaks at RSS-coding sequence junctions. The V(D)J recombinase is composed of two molecules each of two different proteins called RAG1 and RAG2. These proteins are encoded by lymphoid-specific genes, called **recombination-activating gene 1** and **recombination-activating gene 2** (*RAG1* and *RAG2*), respectively. This RAG1/RAG2 complex is required for V(D)J recombination, but only RAG1 possesses catalytic activity. The RAG2 protein binds to hypermethylated H3K4 sites in chromatin and associates with and activates RAG1. The RAG1 protein, in a manner similar to a bacterial restriction endonuclease, recognizes the DNA sequence at the junction between a heptamer and a coding segment, but

it is enzymatically active only when complexed with the RAG2 protein. RAG1 then makes a nick (on one DNA strand) between the coding end and the heptamer. The released 3′ OH of the coding end then attacks a phosphodiester bond on the other DNA strand, forming a covalent hairpin. The signal end (including the heptamer and the rest of the RSS) does not form a hairpin and is generated as a blunt double-strand DNA terminus that undergoes no further processing. This double-strand break results in a closed hairpin of one coding segment being held in apposition to the closed hairpin of the other coding end and two blunt recombination signal ends being placed next to each other. RAG1 and RAG2, in addition to generating the double-strand breaks, also hold the hairpin ends and the blunt ends together before the modification of the coding ends and the process of ligation begins.

RAG genes are expressed only in developing B and T cells. RAG proteins are produced mainly in the G0 and G1 stages of the cell cycle and are inactivated in proliferating cells. It is thought that limiting DNA cleavage and recombination to the G0 and G1 stages minimizes the risk for generating inappropriate DNA breaks during DNA replication or during mitosis. Mice without functional *Rag1* or *Rag2* genes (*Rag* knockout mice) fail to develop B or T lymphocytes, and *RAG1* or *RAG2* mutations are a cause of SCID, in which patients lack B and T lymphocytes (see Chapter 21).

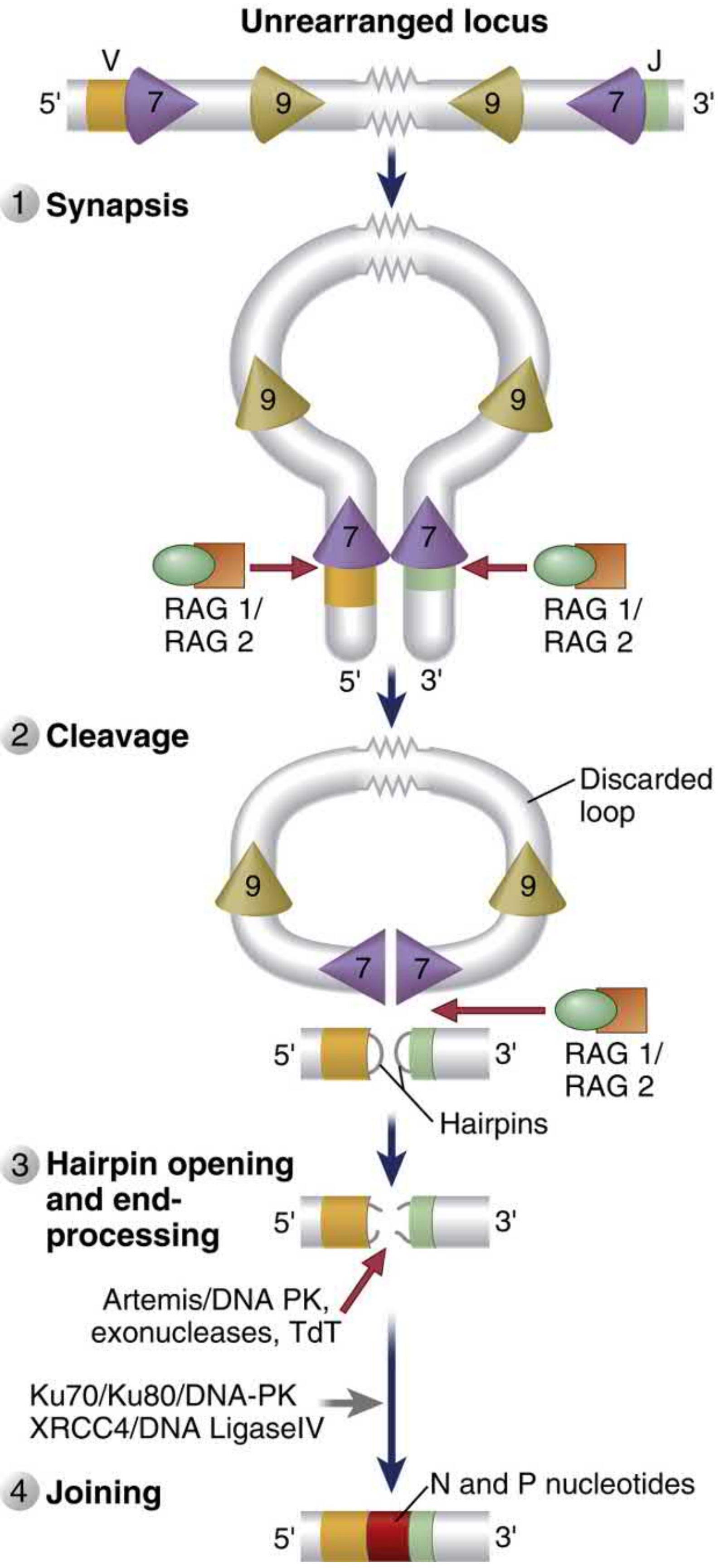

Unrearranged locus
V
J
5'
7
9
9
7
3'
1 Synapsis
9
9
7
7
RAG 1/
RAG 2
RAG 1/
RAG 2
5'
3'
2 Cleavage
Discarded
loop
9
9
7
7
5'
3'
RAG 1/
RAG 2
Hairpins
3 Hairpin opening
and end-
processing
5'
3'
Artemis/DNA PK,
exonucleases, TdT
Ku70/Ku80/DNA-PK
XRCC4/DNA LigaseIV
4 Joining
N and P nucleotides
5'
3'

FIGURE 8.10 Sequential events during V(D)J recombination.Synapsis and cleavage of DNA at the heptamer/coding segment boundary are mediated by Rag-1 and Rag-2. The coding end hairpin is opened by the ARTEMIS endonuclease, and broken ends are repaired by the nonhomologous end joining machinery present in all cells. Note that the two strands of DNA are shown in the hairpins but not in other schematic illustrations of genes.

3. ***Hairpin opening and end processing.*** After the formation of double-strand breaks, hairpins must be opened at the coding junctions, and nucleotides may be added to or removed from the coding ends to create even greater diversification. **ARTEMIS** is an endonuclease that opens up the hairpins at the coding ends. In the absence of ARTEMIS, hairpins cannot be opened and mature T and B cells cannot be generated. Mutations in *ARTEMIS* are a rare cause of SCID, similar to patients with *RAG1* or *RAG2* mutations (see Chapter 21). A lymphoid-specific enzyme, called **terminal deoxynucleotidyl transferase (TdT)**, adds nucleotides to broken DNA ends and will be discussed later in the context of junctional diversity.
4. ***Joining.*** The broken coding ends as well as the signal ends (the ends that terminate in noncoding RSS sequences) are brought together and ligated by a double-strand break repair process found in all cells that is called nonhomologous end joining. A number of ubiquitous proteins participate in nonhomologous end joining. KU70 and KU80 are DNA end-binding proteins that bind to the breaks and recruit the catalytic subunit of DNA-dependent protein kinase (DNA-PK), a DNA repair enzyme. Mutations affecting DNA-PK result in a failure to produce mature B

and T lymphocytes, causing SCID in mice and humans (see Chapter 21). DNA-PK also phosphorylates and activates ARTEMIS, which, as mentioned before, is involved in end processing. Ligation of the processed broken ends is mediated by DNA ligase IV and XRCC4, the latter being a noncatalytic but essential subunit of the ligase.

Generation of Diversity in B and T Cells

The diversity of the B and T cell repertoires is created by random combinations of germline gene segments being joined together and by the addition or deletion of sequences at the junctions between these segments. Several genetic mechanisms contribute to this diversity (Fig. 8.11 and Table 8.1).

- ***Combinatorial diversity. Different combinations of gene segments united by V(D)J recombination encode antigen receptors with different antigen-binding sites.*** The maximum possible number of combinations of these gene segments is the product of the numbers of V, J, and (if present) D gene segments at each antigen receptor locus. Therefore, the amount of combinatorial diversity that can be generated at each locus reflects the number of germline V, J, and D gene segments at that locus. After synthesis of antigen receptor proteins, combinatorial diversity is further enhanced by the juxtaposition of two different, randomly generated V regions (i.e., V_H and V_L in Ig molecules and $V\alpha$ and $V\beta$ in TCR molecules). Therefore, the total combinatorial diversity is theoretically the product of the combinatorial diversity of each of the two associating chains. The actual degree of combinatorial diversity in the expressed Ig and TCR repertoires in any individual is likely to be considerably less than the theoretical maximum. This is because not all combinations of gene segments are equally likely to occur and not all pairings of Ig heavy and light chains or TCR α and β chains may form functional antigen receptors.

Importantly, because the numbers of V, D, and J segments in each locus are limited (see Table 8.1), the maximum possible numbers of combinations are on the order of 1 to 3 million (the upper limit of combinatorial diversity). This is much less than the actual diversity of antigen receptors in mature lymphocytes.

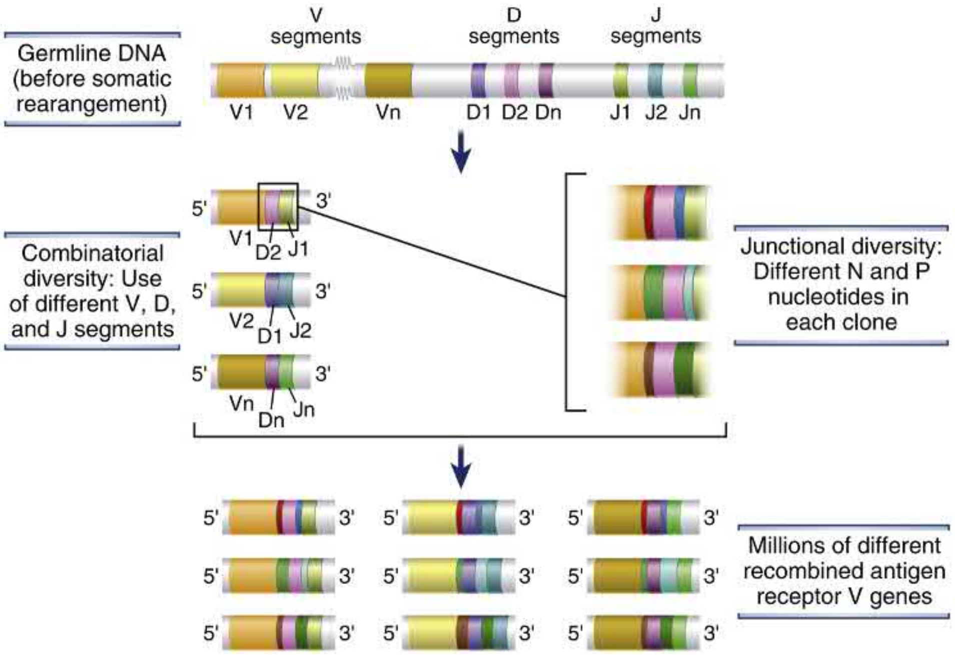

Germline DNA (before somatic rearrangement)
V segments
D segments
J segments
V1
V2
Vn
D1
D2
Dn
J1
J2
Jn
Combinatorial diversity: Use of different V, D, and J segments
5'
3'
V1
D2
J1
V2
D1
J2
Vn
Dn
Jn
Junctional diversity: Different N and P nucleotides in each clone
Millions of different recombined antigen receptor V genes

FIGURE 8.11 Diversity of antigen receptor genes. The figure shows the two mechanisms that give rise to the diversity of antigen receptors—combinations of different V, D, and J gene segments and addition or removal of nucleotides at the joints. Although different V-D-J combinations and addition of N/P nucleotides are shown separately, to emphasize the different contributions of combinatorial and junctional diversity, both processes occur at the same time during rearrangement of gene segments. Not shown is removal of nucleotides, which also occurs simultaneously and contributes to more junctional diversity. The figure illustrates a few distinct antigen receptor genes produced by these mechanisms, but the total diversity is enormous (see Table 8.1).

- *Junctional diversity. The largest contribution to the diversity of antigen receptors is made by the addition or removal of nucleotides at the junctions of the V and D, D and J, or V and*

J segments at the time these segments are joined. One way in which this can occur is if endonucleases remove nucleotides from the germline sequences at the ends of the recombining gene segments. In addition, new nucleotide sequences, not present in the germline, may be added at junctions (Fig. 8.12). As described earlier, coding segments (e.g., V and J gene segments) that are cleaved by RAG1 form hairpin loops. Each hairpin DNA is nicked (cleavage of a single DNA strand) asymmetrically by the enzyme ARTEMIS so that the hairpin is opened and one DNA strand is then longer than its complementary strand. The shorter strand has to be extended with nucleotides complementary to the longer strand before the two coding segments can be ligated. The longer strand thus serves as a template for the addition of short lengths of new nucleotides called P nucleotides, and this process introduces new sequences at the V-D-J junctions. Another mechanism of junctional diversity is the random addition of up to 20 non-template-encoded nucleotides called N nucleotides (see Fig. 8.12). N region diversification is more common in Ig heavy chains and in TCR β and γ chains than in Ig κ or λ chains. This addition of new nucleotides is mediated by the enzyme TdT. In mice rendered deficient in TdT by gene knockout, the diversity of B and T cell repertoires is substantially less than in normal mice. The addition of P nucleotides and N nucleotides at the recombination sites may introduce frameshifts, theoretically generating termination codons in two of every three joining events (if the total number of added bases is not a multiple of three). These genes cannot produce functional proteins, but such inefficiency is the price that is paid for generating diversity.

Because of junctional diversity, antibody and TCR molecules show the greatest variability at the junctions of V and C regions, which form the third hypervariable region, or CDR3 (see Fig. 8.5) **.** In fact, because of junctional diversity, the numbers of different amino acid sequences that are present in the CDR3 regions of Ig and TCR molecules are much greater than the numbers that can be encoded by germline gene segments. As expected, the CDR3 regions of Ig and TCR molecules are also the most important portions of these molecules for determining the specificity of antigen binding (see Chapters 5 and 7).

TABLE 8.1

	Immunoglobulin			T Cell Receptor αβ		T Cell Receptor γδ	
Mechanism	**Heavy Chain**	**κ**	**λ**	**α**	**β**	**γ**	**δ**
Variable (V) segments	45	35	30	45	50	5	2
Diversity (D) segments	23	0	0	0	2	0	3
D segments read in all three reading frames	Rare	—		—	Often	—	Often
N region diversification	V-D, D-J	None		V-J	V-D, D-J	V-J	V-D1, D1-D2, D1-J
Joining (J) segments	6	5	4	55	12	5	4
Total potential repertoire with junctional diversity	~10^{11}		—	~10^{16}		~10^{18}	

The potential number of antigen receptors with junctional diversity is much greater than the number that can be generated only by combinations of V, D, and J gene segments. The calculated figures for lymphocyte repertoire magnitudes should be considered very gross approximations. The calculations for the Ig repertoire do not account for the phenomenon of somatic hypermutation, which will be discussed in Chapter 12.

Although the theoretical limit to the number of Ig and TCR proteins that can be produced is enormous (see Table 8.1), the actual number of antigen receptors on B or T cells expressed in each individual at any one point in time is probably on the order of only 10^7 or 10^8. This is largely a reflection of the finite number of lymphocytes that an individual can accommodate at any time.

There are a number of clinical applications of our knowledge of junctional diversity. One is the determination of the clonality of lymphoid tumors that arise from B or T cells. This laboratory test is used to identify monoclonal tumors of lymphocytes and to distinguish tumors from polyclonal proliferations. Because every lymphocyte clone expresses a unique antigen receptor CDR3 region, the sequence of nucleotides at the V(D)J recombination site serves as a specific marker for each clone. Thus, by determining the sequence of the junctional regions of Ig or TCR genes in different B or T cell proliferations, one can establish whether these lesions arose from a single clone (indicating a tumor) or independently from different clones (implying nonneoplastic proliferation of lymphocytes). The same method may be used to identify small numbers of tumor cells in the blood or tissues. Other applications include analyzing CDR3 sequences of T and B cells in infectious and autoimmune diseases and tumor-infiltrating lymphocytes in cancers to determine expansion of some clones during immune responses (although this method does not indicate what antigens the clones might recognize).

With this background, we proceed to a discussion of B lymphocyte development and then the maturation of T cells.

B Lymphocyte Development

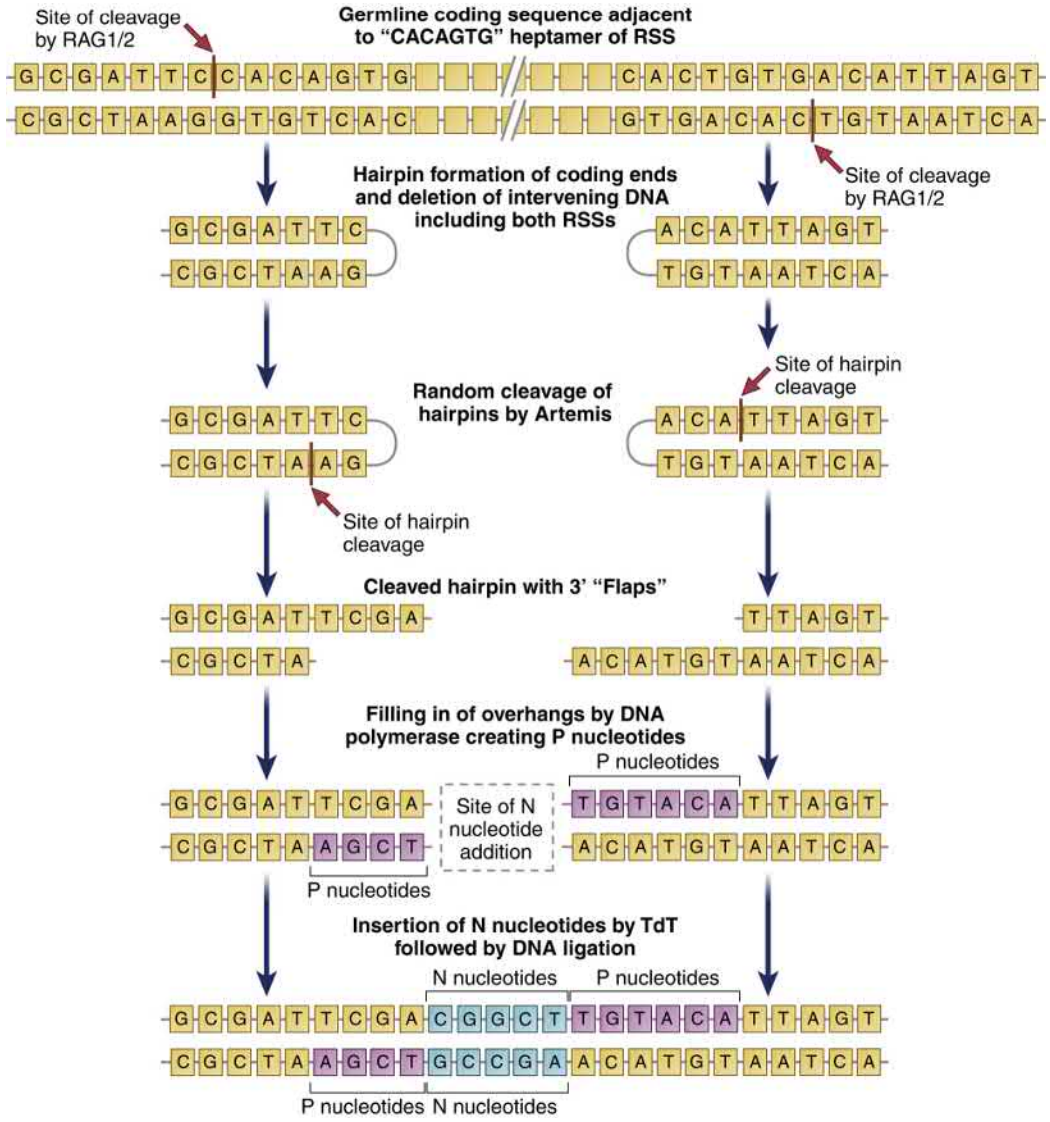
The steps in the maturation of B lymphocytes are the rearrangement and expression of Ig genes in a precise order, selection and proliferation of developing B cells at the pre-antigen receptor checkpoint, and selection of the mature B cell repertoire. Before birth, B lymphocytes develop from committed precursors in the fetal liver, and after birth, B cells are generated in the bone marrow. The majority of B lymphocytes arise from adult bone marrow progenitors that initially do not express Ig. These precursors develop into immature B cells that express membrane-bound IgM molecules and then leave the bone marrow to mature further, mainly in the spleen. IgM-expressing B cells that emerge from the bone marrow into the peripheral blood in humans are called **transitional B cells,** and they exist in three distinguishable stages of maturation. At the late transitional B cell stages, self-reactive cells are inactivated or eliminated in the spleen and other secondary lymphoid organs (see Chapter 15). Eventually the surviving B cells mature into follicular B cells that express IgM and IgD on the surface and acquire the ability to recirculate and populate all secondary lymphoid organs. These follicular B cells home to lymphoid follicles in secondary lymphoid organs and are able to recognize and respond to foreign antigens. The development of a mature B cell from a lymphoid progenitor is estimated to take 2 to 3 days in humans.

Stages of B Lymphocyte Development

During their maturation, cells of the B lymphocyte lineage go through sequential stages, each characterized by distinct cell surface markers and a specific pattern of Ig gene expression (Fig. 8.13) **.** The major stages and the events in each are described next.

The Pro-B and Pre-B Stages of B Cell Development

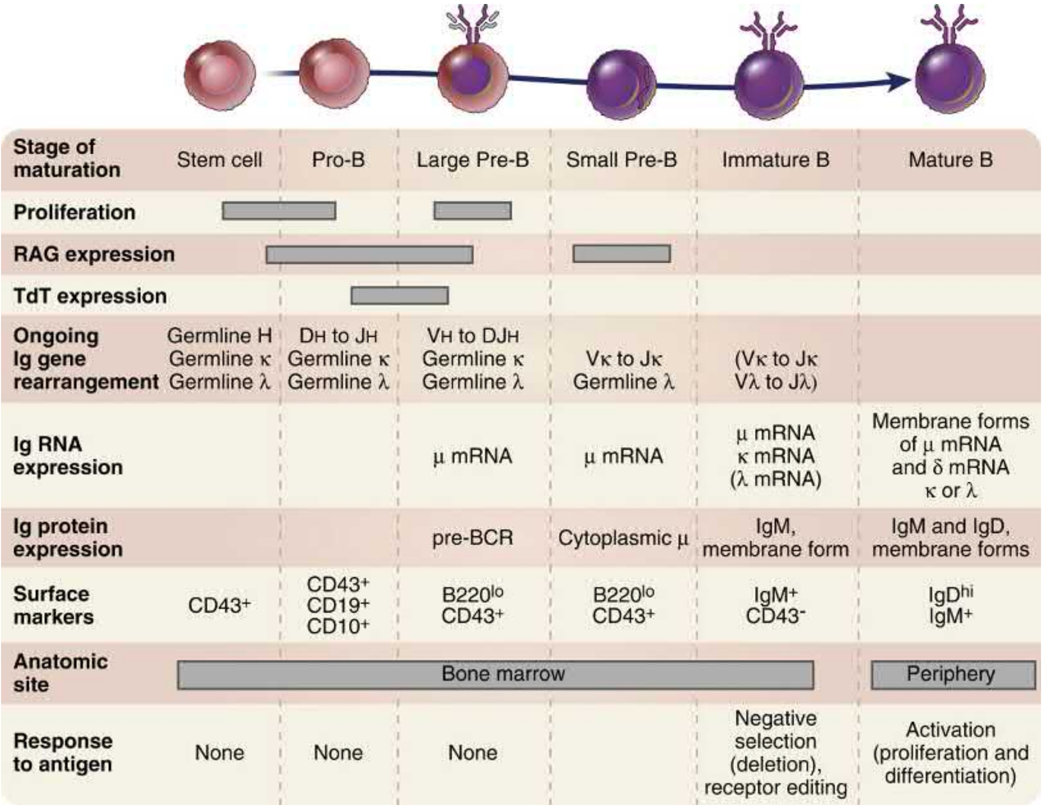
The earliest bone marrow cell committed to the B cell lineage is the pro-B cell. Pro-B cells do not produce Ig, but they can be distinguished from other immature cells by the expression of B lineage–restricted surface molecules such as CD19 and CD10. RAG1 and RAG2 proteins are first expressed at this stage, and the first recombination of Ig genes occurs at the heavy chain locus. This recombination brings together one D and one J gene segment, with deletion of the intervening DNA (Fig. 8.14A). The D segments that are 3′ of the rearranged D segment, and the J segments that are 5′ of the rearranged J segment are deleted by this recombination (e.g., D1 and J2 to J6 in Fig. 8.14A). After the D-J recombination event, one of the many 5′ V gene segments is joined to the DJ unit, giving rise to a rearranged VDJ exon. At this stage, all V and D segments between the rearranged V and D gene segments are also deleted. V-to-DJ recombination at the Ig heavy chain locus occurs only in committed B lymphocyte precursors and is a critical event in Ig expression because only the rearranged V gene is subsequently transcribed. The TdT enzyme, which catalyzes the addition of junctional N nucleotides (see Fig. 8.12), is expressed most abundantly during the pro-B stage when VDJ recombination occurs at the IgH locus, and levels of TdT decrease before light chain gene V-J recombination is complete. Therefore, junctional diversity attributed to addition of N nucleotides is more prominent in rearranged heavy chain genes than in light chain genes.



Site of cleavage by RAG1/2
Germline coding sequence adjacent to "CACAGTG" heptamer of RSS
G C G A T T C C A C A G T G
C A C T G T G A C A T T A G T
C G C T A A G G T G T C A C
G T G A C A C T G T A A T C A
Site of cleavage by RAG1/2
Hairpin formation of coding ends and deletion of intervening DNA including both RSSs
Random cleavage of hairpins by Artemis
Site of hairpin cleavage
Site of hairpin cleavage
Cleaved hairpin with 3' "Flaps"
Filling in of overhangs by DNA polymerase creating P nucleotides
P nucleotides
Site of N nucleotide addition
P nucleotides
Insertion of N nucleotides by TdT followed by DNA ligation
N nucleotides
P nucleotides
P nucleotides
N nucleotides

FIGURE 8.12 Junctional diversity.During the joining of different gene segments, addition or removal of nucleotides may lead to the generation of novel nucleotide and amino acid sequences at the junction. Nucleotides (P sequences) may be added to asymmetrically cleaved hairpins in a templated manner. Other nucleotides (N regions) may be added to the sites of V-D, V-J, or D-J junctions in a nontemplated manner by the action of the enzyme terminal deoxynucleotidyl transferase *(TdT).* These additions generate new

sequences that are not present in the germline. *RSSs,* Recombination signal sequences.



Stage of maturation	Stem cell	Pro-B	Large Pre-B	Small Pre-B	Immature B	Mature B
Proliferation	▬	▬	▬			
RAG expression		▬	▬	▬		
TdT expression		▬	▬			
Ongoing Ig gene rearrangement	Germline H Germline κ Germline λ	D_H to J_H Germline κ Germline λ	V_H to DJ_H Germline κ Germline λ	V_κ to J_κ Germline λ	(V_κ to J_κ V_λ to J_λ)	
Ig RNA expression			μ mRNA	μ mRNA	μ mRNA κ mRNA (λ mRNA)	Membrane forms of μ mRNA and δ mRNA κ or λ
Ig protein expression			pre-BCR	Cytoplasmic μ	IgM, membrane form	IgM and IgD, membrane forms
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	$B220^{lo}$ $CD43^+$	IgM^+ $CD43^-$	IgD^{hi} IgM^+
Anatomic site	Bone marrow					Periphery
Response to antigen	None	None	None		Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

FIGURE 8.13 Stages of B cell maturation.Events corresponding to each stage of B cell maturation from a bone marrow stem cell to a mature follicular B lymphocyte are illustrated. “Ongoing” Ig gene rearrangement implies that at this stage these are the gene segments being actively rearranged. “Germline” implies that the gene locus in question had not been altered by V(D)J recombination. Several surface markers in addition to those shown have been used to define distinct stages of B cell maturation. Maturation of B-1 and other B cell subsets is not shown. *Ig,* Immunoglobulin.

The heavy chain C region exons remain separated from the newly created VDJ exon by DNA containing the distal J segments and the J-C intron. The rearranged Ig heavy chain gene is transcribed to produce a primary transcript that includes the rearranged VDJ exon and the Cμ exons. The nuclear RNA of the rearranged heavy chain gene is cleaved downstream of one of two consensus polyadenylation sites, and multiple adenine nucleotides, called poly-A tails, are added to the 3′ end. This nuclear RNA undergoes splicing, an RNA processing event in which the introns are removed and exons joined together. In the case of the μ RNA, introns between the leader exon and the VDJ exon, between the VDJ exon and the first exon of the Cμ locus, and between each of the subsequent constant region exons of Cμ are removed, thus giving rise to a spliced mRNA encoding the μ heavy chain. If the mRNA is derived from an Ig locus at which rearrangement was productive (in the correct reading frame), translation of the rearranged μ heavy chain mRNA leads to synthesis of the μ protein. Approximately half of all pro-B cells make productive rearrangements at the IgH locus on at least one chromosome and can thus go on to synthesize the μ heavy chain protein. Only cells that make productive rearrangements survive and differentiate further.

Once a productive Ig μ rearrangement is made, a cell ceases to be called a pro-B cell and has differentiated into the pre-B stage. **Pre-B cells** are developing B lineage cells that express the Ig μ protein but have yet to rearrange their light chain loci. The pre-B cell expresses the μ heavy chain on the cell surface, in association with other proteins, in a complex called the pre-BCR, which has several important roles in B cell maturation.

The Pre-B Cell Receptor

Complexes of μ heavy chain, surrogate light chains, and the signal-transducing proteins Igα and Igβ form the pre-antigen receptor of the B lineage, known as the pre-BCR. The μ heavy chain associates with the λ5 and Vpre-B proteins, also called surrogate light chains because they are structurally homologous to κ and λ light chains but are invariant (i.e., they are identical in all pre-B cells) and are synthesized only in pro-B and pre-B cells (Fig. 8.15A). This receptor associates with the signaling molecules Igα and Igβ (also known as CD79A and CD79B) to form the pre-BCR complex, similar to the BCR complex in mature B cells (see Chapter 7). Signals from the pre-BCR allow the cells to survive and are responsible for the largest proliferative expansion of B lineage cells during B cell development. During this proliferation, synthesis of RAG proteins is transiently shut off, so Ig gene rearrangement is temporarily halted. It is not known if the pre-BCR recognizes any ligand; the consensus view is that this receptor functions in a ligand-independent manner and that it is activated by the process of assembly. The importance of pre-BCRs is illustrated by studies of knockout mice and rare cases of human deficiencies of these receptors. For instance, in mice, engineered deletion of the gene encoding the μ chain or one of the surrogate light chains results in markedly reduced numbers of mature B cells because development is blocked at the pro-B stage.

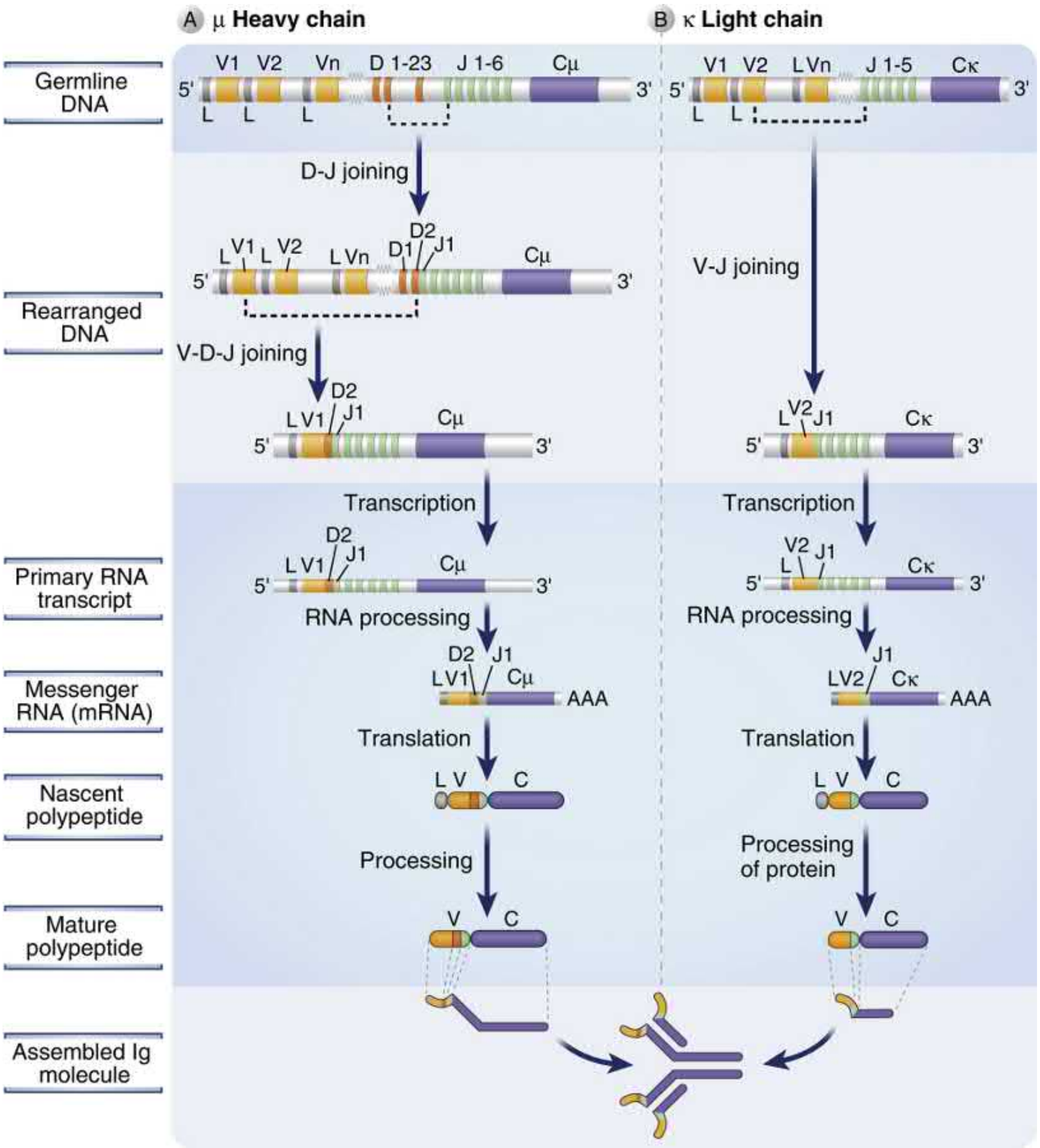
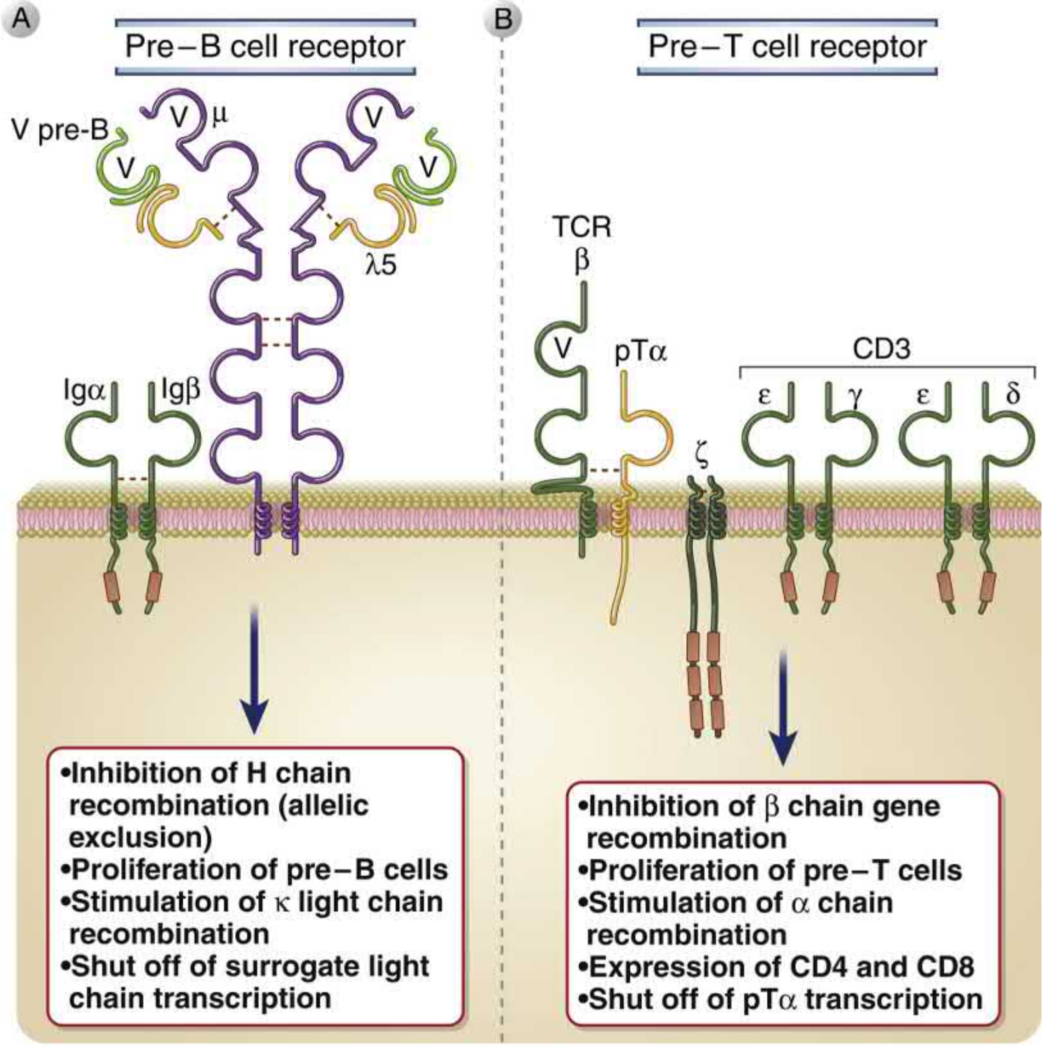

A μ Heavy chain
B κ Light chain
Germline DNA
V1 V2 Vn D 1-23 J 1-6 Cμ
5' 3'
L L L
V1 V2 L Vn J 1-5 Cκ
5' 3'
L L
D-J joining
D2
L V1 L V2 L Vn D1 J1 Cμ
5' 3'
Rearranged DNA
V-D-J joining
V-J joining
D2
L V1 J1 Cμ
5' 3'
L V2 J1 Cκ
5' 3'
Transcription
Transcription
Primary RNA transcript
D2
L V1 J1 Cμ
5' 3'
V2 J1
L Cκ
5' 3'
RNA processing
RNA processing
Messenger RNA (mRNA)
D2 J1
L V1 Cμ AAA
J1
L V2 Cκ AAA
Translation
Translation
Nascent polypeptide
L V C
L V C
Processing
Processing of protein
Mature polypeptide
V C
V C
Assembled Ig molecule

FIGURE 8.14 Immunoglobulin heavy and light chain gene recombination and expression. The sequence of DNA recombination and gene expression events is shown for the immunoglobulin (Ig) μ heavy chain **(A)** and the Ig κ light chain **(B).** In the example shown in **A,** the V region of the μ heavy chain is encoded by the rearranged V1, D2, and J1 gene segments. In the example shown in **B,** the V region of the κ chain is encoded by the V2 and J1 gene segments.



- Inhibition of H chain recombination (allelic exclusion)
- Proliferation of pre–B cells
- Stimulation of κ light chain recombination
- Shut off of surrogate light chain transcription

- Inhibition of β chain gene recombination
- Proliferation of pre–T cells
- Stimulation of α chain recombination
- Expression of CD4 and CD8
- Shut off of pTα transcription

FIGURE 8.15 Pre-B cell and pre-T cell receptors. The pre-B cell receptor *(pre-BCR)* **(A)** and the pre-T cell receptor *(pre-TCR)* **(B)** are expressed during the pre-B cell and pre-T cell stages of maturation, respectively, and the receptors share similar structures and functions. The pre-BCR is composed of the μ heavy chain and an invariant surrogate light chain. The surrogate light chain is composed of two proteins: the V pre-B protein, which is homologous to a light chain V domain, and a λ5 protein that is covalently attached to the μ heavy chain by a disulfide bond. The pre-TCR is composed of the TCR β chain

and the invariant pre-T α *(pTα)* chain. The pre-BCR is associated with the Igα and Igβ signaling molecules that are also part of the BCR complex in mature B cells (see Chapter 9), and the pre-TCR associates with the CD3 and ζ proteins that are also part of the TCR complex in mature T cells (see Chapter 7). *Ig,* Immunoglobulin.

The expression of the pre-BCR is the first checkpoint in B cell maturation. Numerous signaling molecules linked to the pre-BCR (and the BCR in mature B cells) are required for cells to successfully negotiate the pre-BCR–mediated checkpoint at the pro-B to pre-B cell transition. A kinase called Bruton tyrosine kinase (BTK) is activated downstream of the pre-BCR and is required for delivery of signals from this receptor that mediate survival, proliferation, and maturation at and beyond the pre-B cell stage. In humans, mutations in the *BTK* gene result in the disease called **X-linked agammaglobulinemia (XLA),** which is characterized by a failure of B cell maturation (see Chapter 21). In a mouse strain called Xid (for X-linked immunodeficiency), mutations in *btk* result in a less severe B cell defect because murine pre-B cells express a second Btk-like kinase called Tec that partially compensates for the defective Btk. Other molecules upstream and downstream of the BTK signaling pathway that are required at this checkpoint include the μ heavy chain gene, the *λ*5 gene, Ig*α*, Igβ, SYK, the BLNK/SLP65 signaling adaptor, and the p85 subunit of PI3-kinase. Mutations of these genes are the causes of rare cases of autosomal recessive agammaglobulinemia (see Chapter 21).

The pre-BCR regulates further rearrangement of Ig genes in two ways. First, if a μ protein is produced from the recombined heavy chain locus on one chromosome and forms a pre-BCR, this receptor signals to irreversibly inhibit rearrangement of the Ig heavy chain locus on the other chromosome. If the first rearrangement is nonproductive, the heavy chain allele on the other chromosome can complete VDJ rearrangement at the IgH locus. Thus, in any B cell clone, one heavy chain allele is productively rearranged and expressed and the other is either retained in the germline configuration or nonproductively rearranged. As a result, an individual B cell can express an Ig heavy chain protein encoded by only one of the two inherited alleles. This phenomenon is called **allelic exclusion,** and it ensures that every B cell will express a single receptor, thus maintaining clonal specificity. Ig heavy chain allelic exclusion involves changes in chromatin structure in the heavy chain locus that limit accessibility to the V(D)J recombinase. If both alleles undergo nonproductive IgH gene rearrangements, the developing cell cannot produce Ig heavy chains, cannot generate a pre-BCR–dependent survival signal, and undergoes programmed cell death. The second way in which the pre-BCR regulates the production of the antigen receptor is by stimulating κ light chain gene rearrangement. Pre-B cells proliferate first as large pre-B cells, and then shut off surrogate light chain gene expression and become nondividing small pre-B cells that express the μ heavy chain intracellularly. These nondividing cells synthesize RAG proteins and are thus able to rearrange their κ light chain genes. Pre-BCR signals contribute to making the κ light chain locus available to the enzymes that mediate V(D)J recombination. If an in-frame rearrangement occurs at the κ locus, the cell will produce a κ light chain protein, which associates with the previously synthesized μ chain to produce a complete IgM protein. If the κ light chain contributes to a self-reactive BCR (see below) or if the κ locus is not productively rearranged, the cell can rearrange the λ locus and again produce a complete IgM molecule.

DNA recombination in the κ light chain locus occurs in a similar manner as in the Ig heavy chain locus (see Fig. 8.14B). There are no D segments in the light chain loci, and therefore recombination involves only the joining of one V segment to one J segment, forming a VJ exon. This VJ exon remains separated from the C region by an intron, and this separation is retained in the primary RNA transcript. Splicing of the primary transcript results in the removal of the intron between the VJ and C exons and generates an mRNA that is translated to produce the κ or λ protein. In the λ locus, alternative RNA splicing may lead to the use of any one of the four functional Cλ exons, but there is no known biologic difference between the resulting types of λ light chains. Production of a κ protein prevents λ rearrangement, and λ rearrangement occurs only if the κ rearrangements in both the inherited κ chain loci were nonproductive or, more commonly, if the rearranged κ light chain is deleted by receptor editing because it contributes to the formation of a self-reactive BCR, discussed later. As a result, an individual B cell clone can express only one of the two types of light chains; this phenomenon is called light chain isotype exclusion. As in the heavy chain locus, a κ or λ gene is expressed from only one of the two parental chromosomes in any given B cell, and the other inherited locus is excluded. Also, as for heavy chains, if both inherited loci for both κ and λ chains are nonfunctionally rearranged in a developing B cell, that cell fails to receive survival signals that are normally generated by the BCR and dies.

Immature B Cells

The first IgM-expressing cell during B cell development is called an immature B cell. The assembled IgM molecules on immature B cells and in all later stages of development are expressed on the cell surface in association with Igα and Igβ, where they function as specific receptors for antigens. The presence of a complete BCR on the cell surface is essential for a developing B cell to survive. There are survival signals provided by the BCR alone in the absence of any triggering antigen, and these are called tonic signals. Assembly of the complete BCR suffices to activate signaling molecules, including PI3-kinase, that keep the B cell alive. These signals also suppress *RAG* gene expression, thus preventing further Ig gene rearrangement. Immature B cells do not proliferate and differentiate in response to antigens. In fact, if they recognize antigens in the bone marrow with high avidity, which may occur if the B cells express receptors for multivalent self antigens that are present in the bone marrow, the B cells may undergo receptor editing or cell death, as described later. These processes are important for the negative selection of strongly self-reactive B cells.

Immature B cells that are not strongly self-reactive leave the bone marrow and complete their maturation in the spleen and other secondary lymphoid organs.

Subsets of Mature B Cells

B cells in the periphery are made up of distinct subsets that develop from different progenitors (Fig. 8.16) **.** Bone marrow–derived HSCs give rise to the majority of B cells. These cells, also called B-2 cells, rapidly pass through two transitional stages and can commit to development either into **marginal zone B cells** or into **follicular B cells. B-1 cells** represent a distinct lineage that develops from fetal liver–derived HSCs.

Follicular B Cells

Most mature B cells belong to the follicular B cell subset and produce membrane-associated IgD in addition to IgM. Each of these B cells coexpresses μ and δ Ig heavy chains using the same VDJ exon to generate the V domain. In each B cell these heavy chain proteins associate with the same κ or λ light chain to produce two membrane receptors with the same antigen specificity. Each B cell produces a long primary RNA transcript containing the rearranged VDJ unit that encodes the V domain, as well as both the Cμ and Cδ genes (Fig. 8.17). If the primary transcript is cleaved and polyadenylated after the μ exons, after RNA splicing the VDJ exon becomes contiguous with Cμ exons, resulting in the generation of a μ mRNA. If, however, the VDJ complex is not linked to Cμ exons but is spliced to Cδ exons, a δ mRNA is produced. Subsequent translation results in the synthesis of a complete μ or δ heavy chain protein, both containing the same V region and therefore having the same specificity. The precise mechanisms that regulate the choice of polyadenylation or splice acceptor sites, by which the rearranged VDJ is joined to either Cμ or Cδ, are poorly understood, as are the signals that determine when and why a B cell expresses both IgM and IgD rather than IgM alone.

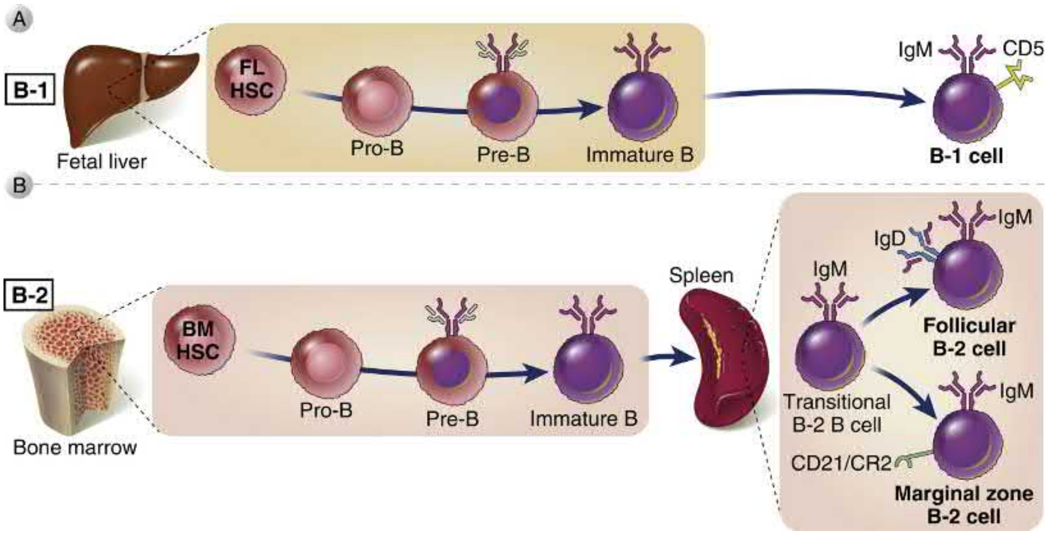

A
B-1
Fetal liver
FL HSC
Pro-B
Pre-B
Immature B
IgM
CD5
B-1 cell
B
B-2
Bone marrow
BM HSC
Pro-B
Pre-B
Immature B
Spleen
IgM
Transitional B-2 B cell
IgD
IgM
Follicular B-2 cell
IgM
CD21/CR2
Marginal zone B-2 cell

FIGURE 8.16 B Lymphocyte subsets. **A,** Most B cells that develop from fetal liver–derived stem cells differentiate into the B-1 lineage. **B,** B lymphocytes that arise from bone marrow precursors after birth give rise to the B-2 lineage. Two major subsets of B lymphocytes are derived from B-2 B cell precursors. Follicular B cells are recirculating lymphocytes; marginal zone B cells are abundant in the spleen in rodents but also can be found in lymph nodes in humans. CD21 is expressed on both follicular and marginal zone B cells, but the levels of this coreceptor are higher on marginal zone B cells. *BM,* bone marrow; *FL,* fetal liver; *HSC,* hematopoietic stem cell; *Ig,* immunoglobulin.

The coexpression of IgM and IgD is accompanied by the ability to recirculate and the acquisition of functional competence, and this is why IgM^+IgD^+ B cells are also called mature B cells. This correlation between expression of IgD and acquisition of functional competence has led to the suggestion that IgD is the essential activating receptor of mature B cells. However, there is no evidence for a functional difference between membrane IgM and membrane IgD. Moreover, knockout of the Ig δ gene in mice does not have a significant impact on the maturation or antigen-induced responses of B cells. Follicular B cells are also often called recirculating B cells because they migrate from one secondary lymphoid organ to the next, and within these organs they reside in follicles (see Chapter 2).

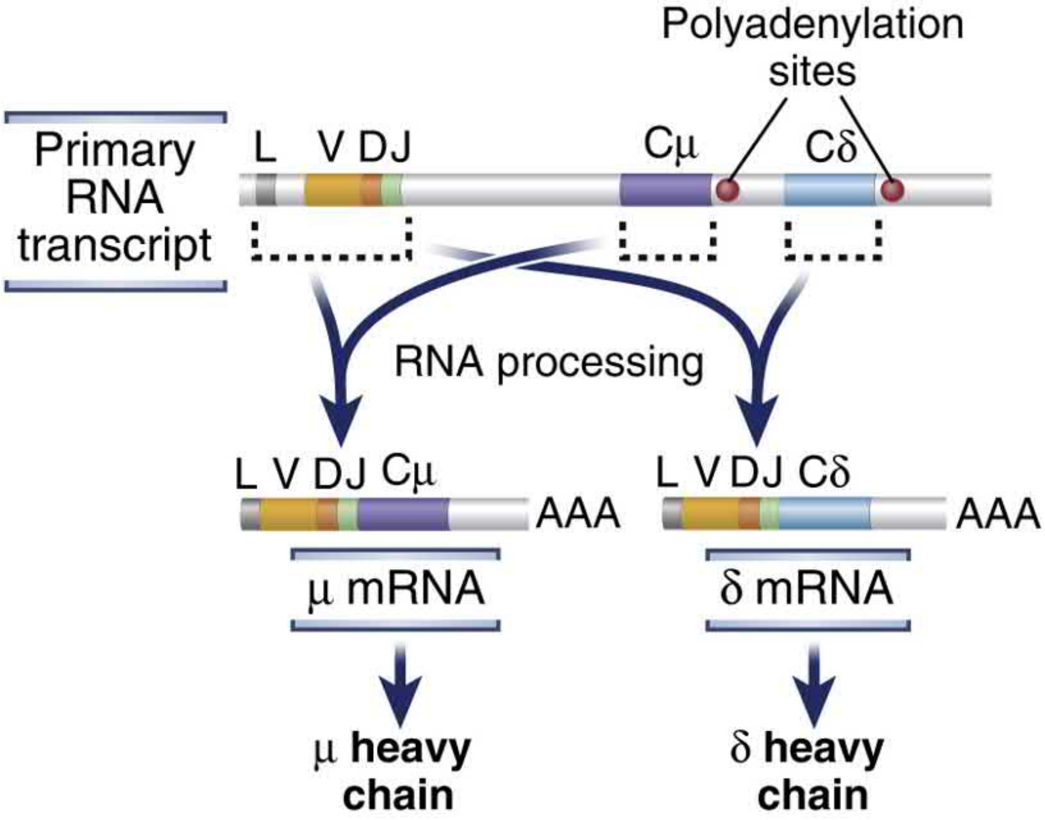

Polyadenylation sites
Primary RNA transcript
L
V DJ
Cμ
Cδ
RNA processing
L V DJ Cμ
AAA
L V DJ Cδ
AAA
μ mRNA
δ mRNA
μ heavy chain
δ heavy chain

FIGURE 8.17 Coexpression of immunoglobulin M and immunoglobulin D.Alternative processing of a primary RNA transcript results in the formation of a μ or δ mRNA. *Dashed lines* indicate the H chain segments that are joined by RNA splicing.

Naive follicular B cells survive for limited periods until they encounter antigen. Follicular B cell survival depends on tonic antigen-independent signals from the BCR as well as stimulation by a cytokine called BAFF (B cell–activating factor of the TNF family, also known as BLyS, for B lymphocyte stimulator), which provides maturation and survival signals through the BAFF receptor. BAFF and a related ligand, APRIL, also can bind to two other receptors, TACI and BCMA, which participate in later stages of B cell activation and differentiation (and will be discussed in Chapter 12). These cytokines are produced by specialized fibroblastic reticular cells and by myeloid cells in lymphoid follicles and in the bone marrow. Naive follicular B cells, like recirculating naive T cells, leave lymph nodes through efferent lymphatics, enter the blood, and return to lymph nodes via high endothelial venules (see Chapter 3).

Mature, naive B cells are responsive to antigens, and unless the cells encounter antigens that they recognize with high affinity and respond to, they die in a few months. In Chapter 12, we will discuss how these cells respond to antigens and how the pattern of Ig gene expression changes during antigen-induced B cell differentiation.

B-1 and Marginal Zone B Cells

A subset of B lymphocytes, called B-1 cells, expresses antigen receptors with limited diversity and may serve roles in humoral immunity that are different from those of follicular B cells. B-1 cells develop from fetal liver–derived HSCs and are best defined in rodents. Most murine B-1 cells express the CD5 molecule. After birth, large numbers of these cells are found as a self-renewing population in the peritoneum and mucosal sites. They develop earlier during ontogeny than follicular and marginal zone B cells, express a relatively limited repertoire of V genes, and exhibit far less junctional diversity than conventional B cells because TdT is not expressed in developing B-1 cells in the fetal liver. B-1 cells spontaneously secrete IgM antibodies that often react with microbial polysaccharides and lipids as well as oxidized lipids produced by lipid peroxidation; most IgM antibodies against ABO blood group antigens are derived from B-1 cells. These antibodies are sometimes called **natural antibodies** because they are present in individuals without overt immunization; it is possible that microbial flora in the gut are the source of antigens that stimulate their production. B-1 cells contribute to rapid antibody production against microbes in particular tissues, such as the peritoneum. At mucosal sites, many IgA-secreting plasma cells in the lamina propria may be derived from B-1 cells. B-1 cells are analogous to γδ T cells in that they both have antigen receptor repertoires of limited diversity and they are both presumed to respond to antigens that are commonly encountered at epithelial interfaces with the external environment. B-1–like cells have been described in humans, but phenotypically this population overlaps with activated B cells, making human B-1 cells harder to define.

Marginal zone B cells are located primarily in the vicinity of the marginal sinus in the spleen and like B-1 cells have limited diversity, respond to polysaccharide antigens, and produce natural antibodies. Marginal zone B cells exist in both mice and humans and express IgM in the absence of IgD and additional surface receptors that distinguish them from follicular B cells. In humans, marginal zone B cells cannot be distinguished from IgM-producing memory cells. In mice, marginal zone B cells exist only in the spleen, whereas in humans, they can be found in the spleen as well as outside follicular areas near the periphery of lymph nodes. Marginal zone B cells respond very rapidly to blood-borne microbes and differentiate into short-lived IgM-secreting plasma cells. These B cells can also participate in T-dependent immune responses.

Selection of the Mature B Cell Repertoire

The repertoire of mature B cells is positively and negatively selected from the pool of immature B cells. As we will see later, positive selection is well defined in T lymphocytes and is responsible for matching the TCRs on newly generated $CD8^+$ and $CD4^+$ T cells with their ability to recognize self class I and class II MHC molecules, respectively. There is no comparable restriction for B cell antigen recognition. Nevertheless, positive selection appears to be a general phenomenon primarily geared to identifying lymphocytes that have successfully completed their antigen receptor gene rearrangement program. Only B cells that express functional membrane Ig molecules can receive tonic BCR-derived signals, which, as described earlier, are required to keep immature B cells alive.

Immature B cells that recognize self antigens with high avidity are often induced to change their specificities by the process of **receptor editing.** Self antigen recognition by immature B cells induces reactivation of *RAG* genes and the rearrangement and production of a new Ig light chain, allowing the cell to express a different (edited) BCR that is not self-reactive. The original VκJκ exon encoding the variable domain of an autoreactive light chain gene is typically deleted and replaced by a new rearrangement involving an upstream Vκ and a downstream Jκ gene segment. If the editing process fails to generate an in-frame productive κ light chain rearrangement on either chromosome, the activated immature B cell may then go on to rearrange the λ light chain first on one chromosome, and if that is nonproductive, then the λ light chain on the other chromosome. Almost all B cells bearing λ light chains are likely derived from immature B cells that were self-reactive and have undergone receptor editing.

If receptor editing fails, the immature B cells that express high-affinity receptors for self antigens and encounter these antigens in the bone marrow or the spleen may die by apoptosis. This process is called **negative selection.** Most negative selection occurs in transitional B cells in the spleen. The antigens mediating negative selection deliver strong signals to IgM-expressing immature B lymphocytes whose receptors happen to be specific for these self antigens. Both receptor editing and deletion are responsible for maintaining B cell tolerance to self antigens that are present in the bone marrow (see Chapter 15).

Once the transition is made to the $IgM^{+}IgD^{+}$ mature B cell stage, antigen recognition leads to proliferation and differentiation, not to receptor editing or apoptosis. As a result, mature B cells that recognize antigens with high affinity in peripheral lymphoid tissues are activated, and this process leads to humoral immune responses. Follicular B cells make most of the helper T cell–dependent antibody responses to protein antigens (see Chapter 12).

T Lymphocyte Development

The development of mature T lymphocytes from committed progenitors involves the sequential rearrangement and expression of TCR genes, cell proliferation, antigen-induced selection, and commitment to phenotypically and functionally distinct subsets (Fig. 8.18) **.** In many ways, this is similar to B cell maturation. However, T cell maturation has some unique features that reflect the specificity of the majority of T lymphocytes for peptide antigens bound to self MHC molecules, and the need for a special microenvironment for selecting cells with this specificity.

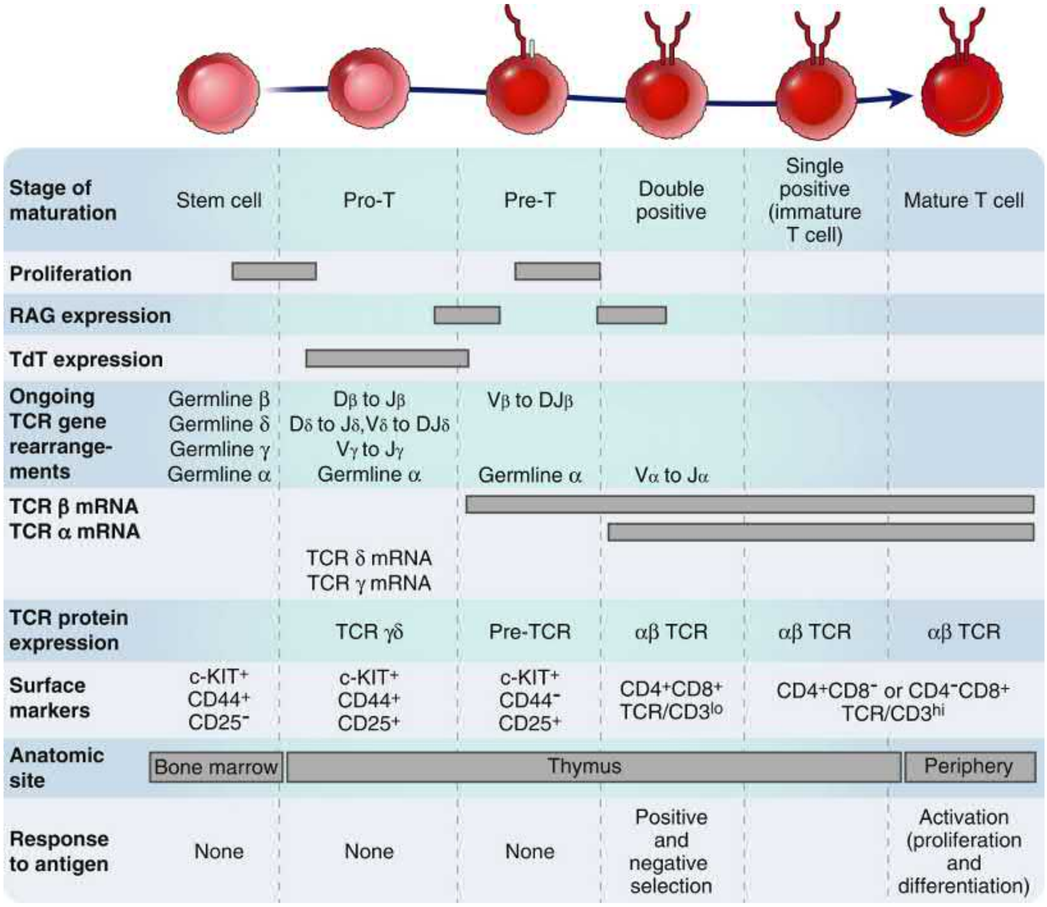

Stage of maturation
Stem cell
Pro-T
Pre-T
Double positive
Single positive (immature T cell)
Mature T cell
Proliferation
RAG expression
TdT expression
Ongoing TCR gene rearrangements
Germline β
Germline δ
Germline γ
Germline α
Dβ to Jβ
Dδ to Jδ, Vδ to DJδ
Vγ to Jγ
Germline α
Vβ to DJβ
Germline α
Vα to Jα
TCR β mRNA
TCR α mRNA
TCR δ mRNA
TCR γ mRNA
TCR protein expression
TCR γδ
Pre-TCR
αβ TCR
αβ TCR
αβ TCR
Surface markers
c-KIT+ CD44+ CD25-
c-KIT+ CD44+ CD25+
c-KIT+ CD44- CD25+
CD4+CD8+ TCR/CD3lo
CD4+CD8- or CD4-CD8+ TCR/CD3hi
Anatomic site
Bone marrow
Thymus
Periphery
Response to antigen
None
None
None
Positive and negative selection
Activation (proliferation and differentiation)

FIGURE 8.18 Stages of T cell maturation. Events corresponding to each stage of T cell maturation from a bone marrow stem cell to a mature T lymphocyte are illustrated. Several surface markers in addition to those shown have been used to define distinct stages of T cell maturation. Thymocytes in the cortex lacking CD4 and CD8 expression are called double negative or DN T cells and are further subdivided into stages based on the expression of two markers, CD25 and CD44. The CD44$^+$CD25$^-$ cells in the cortex that have recently arrived from the bone marrow and are located at the cortico-medullary junction are sometimes called DN1 T cells or pro-T cells and correspond to the pro-B cell stage of B cell maturation. CD44$^+$CD25$^+$ thymocytes are present

in the mid-cortex and often referred to as DN2 T cells; these are committed to becoming T cells, and it is at this stage that TCR β , TCR γ and TCR δ rearrangement commences. $CD44^-CD25^+$ thymocytes are also known as DN3 T cells. An intermediate between DN3 and double-positive (DP) cells has been called the DN4 subset.

Role of the Thymus in T Cell Maturation

The thymus is the major site of maturation of T cells. The thymus involutes with age and is virtually undetectable in postpubertal humans, resulting in a gradual reduction in the output of mature T cells. However, some maturation of T cells continues throughout adult life, as indicated by the successful reconstitution of the immune system in adult recipients of bone marrow transplants. It may be that the remnant of the involuted thymus is adequate for some T cell maturation. Because memory T cells have a long life span (perhaps longer than 20 years in humans) and accumulate with age, the need to generate new T cells decreases as individuals age (see Fig. 2.12).

T lymphocytes originate from precursors that arise in the fetal liver and adult bone marrow and seed the thymus. These precursors are multipotent progenitors that enter the thymus from the blood stream, crossing the endothelium of postcapillary venules in the corticomedullary junction region of the thymus. In mice, immature lymphocytes are first detected in the thymus on the 11th day of the normal 21-day gestation. This corresponds to about week 7 or 8 of gestation in humans. While in the cortex, thymocytes first express γδ and αβ TCRs. The αβ T cells mature into $CD4^+$ class II MHC–restricted or $CD8^+$ class I MHC–restricted T cells as they leave the cortex and enter the medulla. From the medulla, $CD4^+$ and $CD8^+$ single-positive thymocytes exit the thymus into the circulation. We will discuss the maturation of αβ T cells in the following sections and γδ T cells later in the chapter.

The thymic environment provides stimuli that are required for the proliferation and maturation of thymocytes. Many of these stimuli come from thymic cells other than the maturing T cells. Within the cortex, thymic cortical epithelial cells form a meshwork of long cytoplasmic processes around which thymocytes must pass to reach the medulla. Epithelial cells of a distinct type known as medullary thymic epithelial cells are also present in the medulla and may serve a unique role in presenting self antigens for the negative selection of developing T cells (see Chapter 15). Bone marrow–derived dendritic cells (DCs) are present at the corticomedullary junction and within the medulla, and macrophages are present primarily within the medulla. The migration of thymocytes through this anatomic arrangement allows physical interactions between the thymocytes and these other cells that are necessary for the maturation and selection of the T lymphocytes. Epithelial cells and DCs in the thymus express class I and class II MHC molecules. The interactions of maturing thymocytes with these MHC molecules are essential for the selection of the mature T cell repertoire, as we will discuss later.

The movement of cells into and through the thymus is driven by chemokines. The progenitors of thymocytes express the chemokine receptor CCR9. Entry of these precursors into the thymus is dependent on CCR9 binding the chemokine ligand CCL25, which is produced in the thymic cortex. Chemokines such as CCL21 and CCL19, which bind to the CCR7 chemokine receptor on thymocytes, direct the movement of developing T cells from the cortex to the medulla. Eventually, newly formed T lymphocytes, which express the sphingosine-1 phosphate receptor (see Chapter 3), exit the thymic medulla following a gradient of sphingosine-1 phosphate into the blood stream.

Thymic stromal cells, including epithelial cells, secrete IL-7, which was mentioned earlier as a critical lymphopoietic growth factor. The rates of cell proliferation and apoptotic death are extremely high in cortical thymocytes. A single precursor gives rise to many progeny, and 95% of these cells die by apoptosis before reaching the medulla. The cell death is due to a combination of factors, including failure to productively rearrange the TCR β chain gene and thus to fail the pre-TCR/β selection checkpoint (described later), failure to be positively selected by self MHC molecules in the thymus, and self antigen–induced negative selection (see Fig. 8.3).

Stages of T Cell Maturation

During T cell maturation, there is a precise order in which TCR genes are rearranged and in which the TCR and CD4 and CD8 coreceptors are expressed (Fig. 8.19; see also Fig. 8.18) **.** In the mouse fetal thymus, surface expression of the γδ TCR occurs first, 3 to 4 days after precursor cells first arrive, and the αβ TCR is expressed 2 or 3 days later. In human fetal thymuses, γδ TCR expression begins at about 9 weeks of gestation, followed by expression of the αβ TCR at 10 weeks.

Double-Negative Thymocytes

The most immature thymocytes, which are recent arrivals from the bone marrow, contain TCR genes in their germline configuration and do not express TCR, CD3, ζ chains, CD4, or CD8; these cells are called **double-negative (DN) thymocytes** (based on the lack of expression of CD4 and CD8). The majority (>90%) of the DN thymocytes that survive thymic selection processes will ultimately give rise to αβ TCR–expressing, MHC-restricted $CD4^+$ and $CD8^+$ T cells; some DN thymocytes give rise to γδ T cells.

The earliest largely undifferentiated DN thymocytes are located near the cortico-meduallary junction and are beginning to migrate towards the mid-cortex. They are not yet committed to the T lineage. It is at the next stage of cortical thymocyte differentiation in the mid-cortex that DN thymocytes express both CD44 and CD25 (see Fig 8.18), are considered to be at the pro-T cell stage of maturation, and start to rearrange their TCR genes. The RAG1 and RAG2 proteins are first expressed at this stage of T cell development and are required for the rearrangement of TCR genes. In *α*β T cells, Dβ-to-Jβ rearrangements at the TCR β chain locus occur first; these involve either joining of the Dβ1 gene segment to one of the six Jβ1 segments or joining of the Dβ2 segment to one of the six Jβ2 segments (Fig. 8.20A). Vβ-to-DJβ rearrangements occur at the next pre-T stage in the cortex. The DNA sequences between the segments undergoing rearrangement, including D, J, and possibly Cβ1 genes (if Dβ2 and Jβ2 segments are used), are deleted during this rearrangement process. The primary nuclear transcripts of the TCR β genes contain the intron between the recombined VDJβ exon and the relevant Cβ gene (as well as the three additional introns between the four exons that make up each Cβ gene). Poly-A tails are added after cleavage of the primary transcript downstream of consensus polyadenylation sites located 3′ of the Cβ region, and the sequences between the VDJ exon and Cβ are spliced out to form a mature mRNA in which VDJ segments are juxtaposed to the first exon of either of the two Cβ genes (depending on which J segment was selected during the rearrangement process). Translation of this mRNA gives rise to a full-length TCR β protein. The two Cβ genes appear to be functionally interchangeable, and the use of either Cβ gene does not influence the specificity of the TCR. Furthermore, an individual T cell never switches from one C gene to another. The promoters in the 5′ flanking regions of Vβ genes function together with a powerful enhancer that is located 3′ of the Cβ2 gene once rearranged functional V genes are brought close to the C gene by VDJ recombination. This proximity of the promoter to the enhancer is responsible for high-level T cell–specific transcription of the rearranged TCR β chain gene. After the addition and removal of nucleotides during TCR gene rearrangement, the number of new nucleotides in the TCR β chain gene are a multiple of three (in one of the two inherited TCR β loci) in only about half of all developing pre-T cells, and therefore only approximately half of all developing pre-T cells express a TCR β protein. The next step in T cell development selects cells that express the first chain of the antigen receptor and can pass this checkpoint.

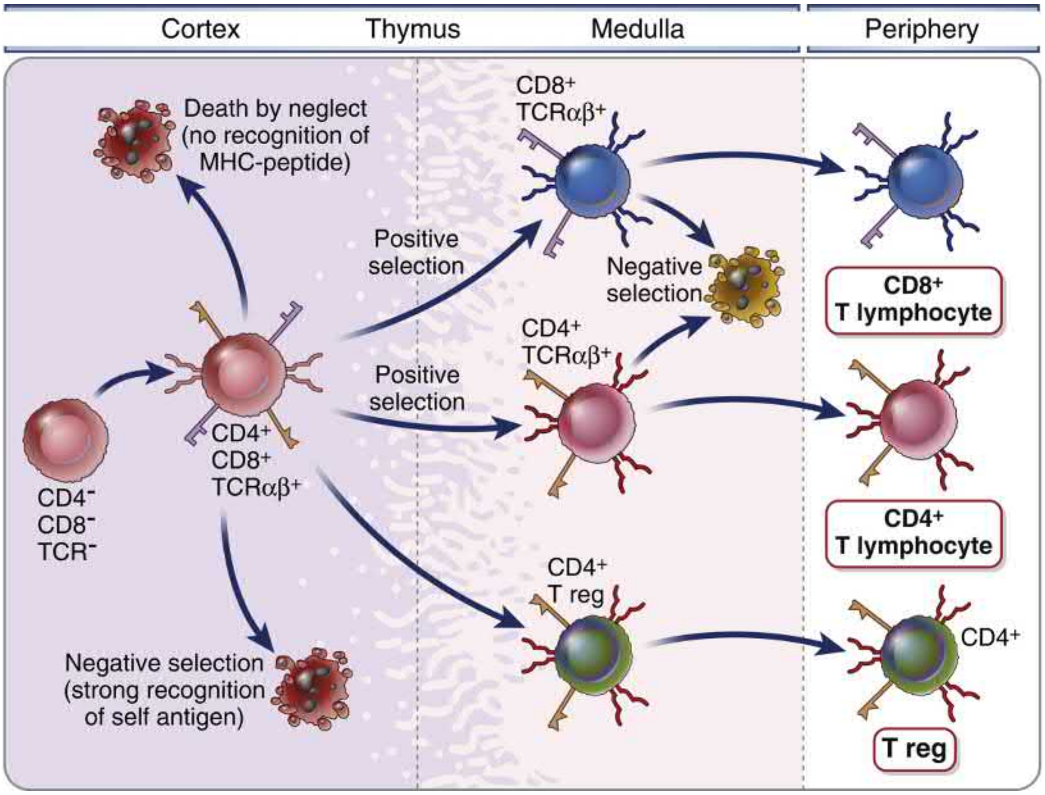

Cortex
Thymus
Medulla
Periphery
Death by neglect (no recognition of MHC-peptide)
CD4⁻ CD8⁻ TCR⁻
CD4⁺ CD8⁺ TCRαβ⁺
Negative selection (strong recognition of self antigen)
Positive selection
Positive selection
CD8⁺ TCRαβ⁺
Negative selection
CD4⁺ TCRαβ⁺
CD4⁺ T reg
CD8⁺ T lymphocyte
CD4⁺ T lymphocyte
CD4⁺
T reg

FIGURE 8.19 An overview of T cell development in the thymus. Precursors of T cells travel from the bone marrow through the blood to the thymus. The progenitors of αβ T cells are double-negative (DN) T cells. In the thymic cortex, these cells begin to express T cell receptors *(TCRs)* and CD4 and CD8 coreceptors. Selection processes eliminate self-reactive T cells in the cortex at the double-positive (DP) stage and also eliminate single-positive (SP) medullary thymocytes. They promote survival of thymocytes whose TCRs bind self major histocompatibility complex *(MHC)* molecules with low affinity. Functional and phenotypic differentiation into $CD4^{+}CD8^{-}$ or $CD8^{+}CD4^{-}$ SP T cells occurs in the medulla, and mature T cells are released into the circulation. Some DP cells differentiate into $CD4^{+}CD8^{-}$ regulatory T cells (Treg; see Chapter 15). The development of γδ T cells is not shown.

Pre-T Cell Receptor

If a productive (i.e., in-frame) rearrangement of the TCR β chain gene occurs in a given double-negative T cell, the TCR β chain is expressed on the cell surface in association with an invariant protein called pre-Tα, which, along with CD3 and ζ proteins, forms the pre-TCR complex (see Fig. 8.15B) **.** The pre-TCR mediates the selection of the developing pre-T cells that have successfully rearranged the β chain of the TCR. The function of the pre-TCR complex in T cell development is similar to that of the surrogate light chain–containing pre-BCR complex in B cell development. Signals from the pre-TCR select cells that have productively rearranged the TCR β chain gene and mediate the survival of these pre-T cells, contribute to the largest proliferative expansion during T cell development, and drive the transition from the double-negative to the double-positive stage of thymocyte development. In addition, these signals inhibit further rearrangement of the TCR β chain locus on the unrearranged allele. This results in β chain allelic exclusion (i.e., mature T cells express an antigen receptor chain from only one of the two inherited β chain loci). As in pre-B cells, it is not known what, if any, ligand the pre-TCR recognizes. Pre-TCR signaling, like pre-BCR signaling, may be initiated in a ligand-independent manner, after the successful assembly of the pre-TCR complex. Pre-TCR signaling is mediated by a number of cytosolic kinases and adaptor proteins that are also linked to TCR signaling (see Chapter 7). The essential function of the pre-TCR complex in T cell maturation has been demonstrated by numerous studies with genetically mutated mice, in which lack of any component of the pre-TCR complex or associated signaling molecules (i.e., the TCR β chain, pre-Tα, CD3, ζ, or LCK) results in a block in the maturation of T cells at the double-negative stage. CD3ε mutations in humans result in SCID (see Chapter 21), whereas mutations in LCK in humans result in the near absence of $CD4^+$ T cells. $CD4^+$ cells are affected more because stronger LCK signals are required for $CD4^+$

than for CD8$^+$ T cell development during positive selection, discussed later.

Double-Positive Thymocytes

At the next stage of T cell maturation, thymocytes express both CD4 and CD8 and are called double-positive T cells. The expression of CD4 and CD8 is essential for subsequent selection events. The rearrangement of the TCR α chain genes and the expression of TCR αβ heterodimers occur in the CD4$^+$CD8$^+$ double-positive population soon after cells cross the pre-TCR checkpoint (see Figs. 8.18 and 8.19). A second wave of *RAG* gene expression late in the pre-T stage promotes TCR α gene recombination. Because there are no D segments in the TCR α locus, rearrangement consists of the joining of only V and J segments (see Fig. 8.20B). The large number of Jα segments permits multiple attempts at productive V-J joining on each chromosome, thereby increasing the probability that a functional αβ TCR will be produced. In contrast to the TCR β chain locus, where production of the protein and formation of the pre-TCR suppress further rearrangement, there is little or no allelic exclusion in the α chain locus. Therefore, productive TCR α rearrangements may occur on both chromosomes, and if this happens, the T cell will express two α chains. In fact, up to 30% of mature peripheral T cells express two different TCRs, with different α chains but the same β chain in each cell. It is possible that only one of the two different TCRs participates in self MHC-driven positive selection, described later. Transcriptional regulation of the α chain gene occurs in a manner similar to that of the β chain. There are promoters 5′ of each Vα gene that have low-level activity and are responsible for high-level T cell–specific transcription when brought close to an α chain enhancer located 3′ of the Cα gene. Unsuccessful rearrangements of the TCR α gene on both chromosomes lead to a failure of positive selection (discussed later). Thymocytes of the αβ T cell lineage that fail to make a productive rearrangement of the TCR α chain gene will die by apoptosis.

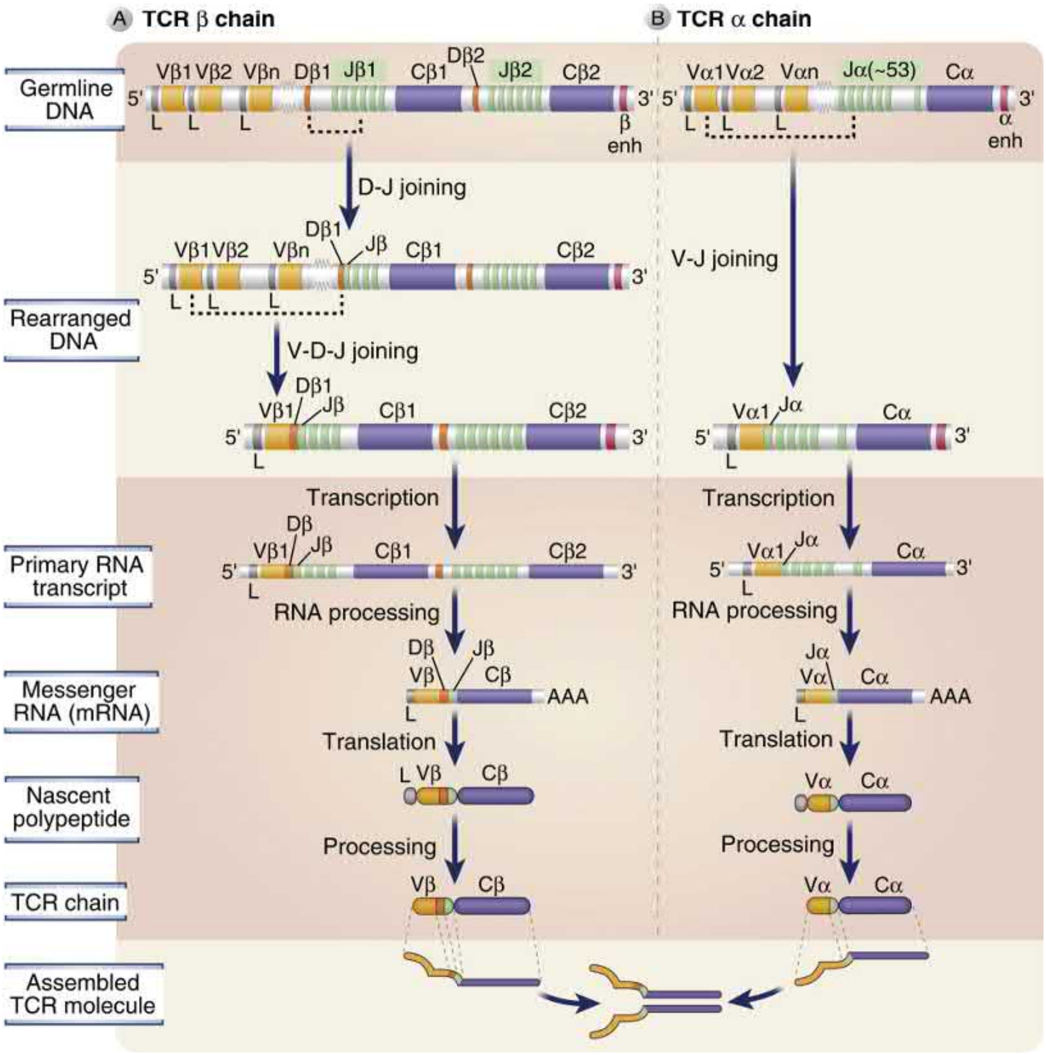

A TCR β chain
B TCR α chain
Germline DNA
Vβ1 Vβ2 Vβn Dβ1 Jβ1 Cβ1 Dβ2 Jβ2 Cβ2
5' 3'
L L L
β enh
Vα1 Vα2 Vαn Jα(~53) Cα
α enh
D-J joining
Rearranged DNA
V-J joining
V-D-J joining
Primary RNA transcript
Transcription
RNA processing
Messenger RNA (mRNA)
AAA
Translation
Nascent polypeptide
Processing
TCR chain
Assembled TCR molecule

FIGURE 8.20 T cell receptor α and β chain gene recombination and expression. The sequence of recombination and gene expression events is shown for the T cell receptor *(TCR)* β chain **(A)** and the TCR α chain **(B).** In the example shown in **A,** the variable (V) region of the rearranged TCR β chain includes the Vβ1 and Dβ1 gene segments and the third J segment in the Jβ1 cluster. The constant (C) region in this example is encoded by the exons of the Cβ1 gene, depicted for convenience as a single exon (though it is actually made up of four exons with three intervening introns). Note that at the TCR β chain locus,

rearrangement begins with D-to-J joining followed by V-to-DJ joining. In humans, 14 Jβ segments have been identified, and not all are shown in the figure. In the example shown in **B,** the V region of the TCR α chain includes the Vα1 gene and the second J segment in the Jα cluster.

This cluster is made up of at least 61 Jα segments in humans; not all are shown here.

TCR *α* gene expression at the double-positive stage leads to the formation of the complete *α*β TCR, which is expressed on the cell surface in association with CD3 and *ζ* proteins. The coordinate expression of CD3 and *ζ* proteins and the assembly of intact TCR complexes are required for surface expression. Rearrangement and expression of the TCR *α* gene at the double-positive stage results in deletion of the TCR δ locus that lies between V segments (common to both *α* and δ loci) and J*α* segments (see Fig. 8.6). As a result, this T cell is no longer capable of becoming a *γ*δ T cell and is committed to the *α*β T cell lineage. The expression of *RAG* genes and further TCR gene recombination cease after this stage of maturation.

Double-positive thymocytes that successfully undergo selection processes go on to mature into CD4 $^{+}$ or CD8 $^{+}$ cells, which are called single-positive T cells. Thus, the stages of T cell maturation in the thymus can be distinguished by the expression of CD4 and CD8 (Fig. 8.21). This phenotypic maturation is accompanied by commitment to different functional programs upon activation in secondary lymphoid organs. CD4^{+} and CD8^{+} T cells acquire unique properties during their maturation; CD4^{+} cells are able to produce different cytokines in response to antigen stimulation and to express effector molecules (such as CD40 ligand) that activate B lymphocytes, DCs, and macrophages, and CD8^{+} cells are able to produce molecules that kill other cells. Mature single-positive thymocytes enter the thymic medulla and then leave the thymus to populate peripheral lymphoid tissues.

Selection Processes in the Maturation of MHC-Restricted αβ T Cells

The selection of developing T cells is dependent on recognition of antigen (peptide-MHC complexes) in the thymus and is responsible for preserving useful cells and eliminating potentially harmful ones. The immature, or unselected, repertoire of T lymphocytes consists of cells whose receptors may recognize any peptide antigen (self or foreign) displayed by any MHC molecule (also self or foreign). In addition, receptors may theoretically be expressed that do not recognize any peptide–MHC molecule complex. In every individual, the only useful T cells are the ones specific for foreign peptides presented by that individual's MHC molecules—that is, self MHC molecules. When double-positive thymocytes first express αβ TCRs, these receptors encounter self peptides (the only peptides normally present in the thymus) displayed by self MHC molecules (the only MHC molecules available to display peptides), mainly on thymic epithelial cells in the cortex. Thus, from the many TCRs of different specificities that are produced, the ones that recognize self MHC have to be preserved, and self peptide recognition has to preserve specificities for foreign antigens. We describe these processes in the next section.

Positive Selection of Thymocytes: Development of the Self MHC–Restricted T Cell Repertoire

Positive selection is the process in which thymocytes whose TCRs bind with low avidity (i.e., weakly) to self peptide–self MHC complexes are stimulated to survive and to differentiate either into CD4$^+$ T cells or CD8$^+$ T cells (see Fig. 8.19) . Double-positive thymocytes are produced without antigenic stimulation and begin to express αβ TCRs. In the thymic cortex, these immature cells encounter epithelial cells that display a variety of self peptides bound to class I and class II MHC molecules. Weak recognition of these self peptide–self MHC complexes promotes the survival of selected T cells. Thymocytes whose receptors do not recognize self

MHC molecules are permitted to die by a default pathway of apoptosis; this phenomenon is called death by neglect (see Fig. 8.19).

During the transition from double-positive to single-positive cells, thymocytes whose TCRs recognize self class I MHC become CD8$^+$ CD4$^-$, and cells with TCRs that recognize self class II MHC become CD4$^+$ CD8$^-$. Thus, these cells become committed to the CD4 or CD8 lineage. Two models have been proposed to explain the process of lineage commitment, as a result of which coreceptors are correctly matched with the TCRs that recognize a specific class of MHC molecules. The stochastic or probabilistic model suggests that the commitment of immature T cells toward either lineage depends on the random probability of a double-positive cell differentiating into a single-positive CD4$^+$ or a CD8$^+$ thymocyte. In this model, a newly generated single-positive CD8$^+$ T cell that has a TCR that can recognize self class I MHC and peptide in the thymus with a low affinity survives because it can engage the CD8 coreceptor, but a newly generated CD8$^+$ T cell whose TCR recognizes only self class II MHC and peptide with low affinity does not survive because its coreceptor does not help trigger the TCR on this T cell. Similarly, only single-positive CD4$^+$ T cells whose TCRs can recognize self class II MHC and peptide (and not self class I MHC and peptide) with low affinity would be assumed to survive positive selection in this stochastic model. An alternative and more widely accepted view is that the process of lineage commitment linked to positive selection is driven by specific signals that instruct the double-positive T cell to become CD4$^+$ or CD8$^+$. According to this instructional model, class I MHC– and class II MHC–restricted TCRs deliver different signals that actively induce expression of the correct coreceptor and shut off expression of the other coreceptor. It is known that double-positive cells go through a stage at which they express high CD4 and low CD8. If the TCR on such a cell is class I MHC–restricted, when it sees the appropriate class I MHC and self peptide, it will receive a weak signal because levels of the CD8 coreceptor are low, and in addition, CD8 associates less well with the LCK tyrosine kinase than does CD4. These weak signals activate transcription factors such as RUNX3 that maintain the $CD8^+$ T cell phenotype by regulating the expression of the CD8 gene and by silencing the CD4 gene. Conversely, if the TCR on the cell is class II MHC–restricted, when it sees class II MHC it will receive a stronger signal because CD4 levels are high and CD4 associates relatively well with LCK. These strong signals activate the transcription factor GATA3, which commits cells toward a CD4 fate, and induces the expression of a repressor called ThPoK, which prevents the expression of lineage defining genes of $CD8^+$ T cells.

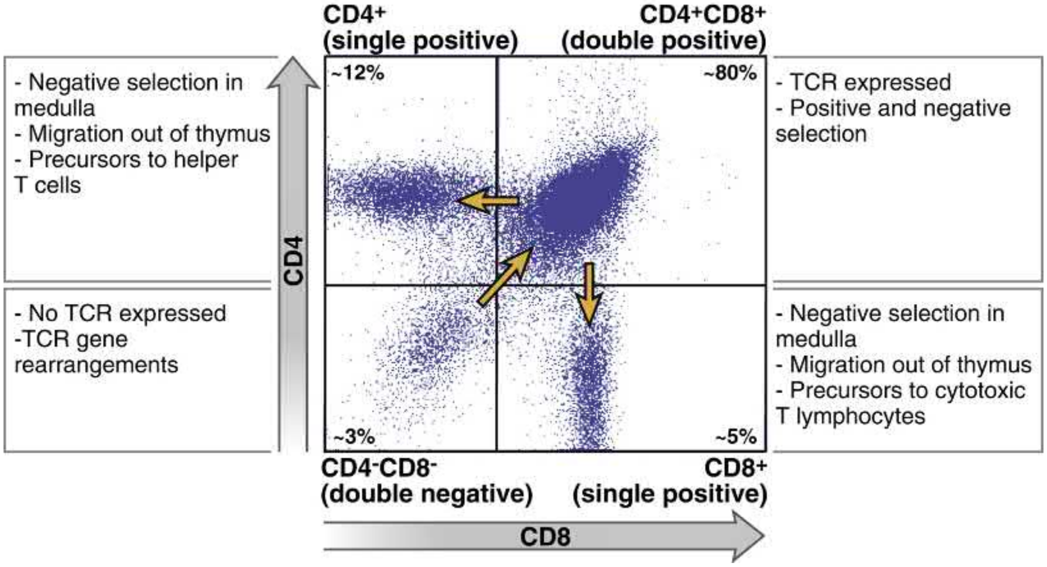


FIGURE 8.21 CD4 and CD8 expression on thymocytes and maturation of T cells in the thymus. The maturation of thymocytes can be followed by changes in expression of the CD4 and CD8 coreceptors. A two-color flow cytometric analysis of thymocytes using anti-CD4 and anti-CD8 antibodies, each tagged with a different fluorochrome, is illustrated. The percentages of all thymocytes contributed by each major population are shown in the four quadrants. The least mature subset is the $CD4^-CD8^-$ (double-negative) cells. *Arrows* indicate the sequence of maturation.

Peptides bound to MHC molecules on thymic epithelial cells play an essential role in positive selection. In Chapter 6, we described how MHC molecules that are expressed on the cell surface always contain bound peptides. These MHC-associated peptides on thymic APCs probably serve two roles in positive selection—first, they promote stable cell surface expression of MHC molecules, and second, they may influence the specificities of the T cells that are selected. It is also clear from a variety of experimental studies that some peptides are better than others in supporting positive selection, and different peptides differ in the repertoires of T cells they select. These results suggest that specific antigen recognition, and not just MHC recognition, has some role in positive selection.

In Chapter 6 we described the unique proteasomal subunit, β5t, that is expressed only in thymic cortical epithelial cells in proteasomes called thymoproteasomes. These functionally altered proteasomes generate unique self peptides that contribute to positive selection on class I MHC expressed on thymic cortical epithelial cells. Similarly, cathepsin V, the human equivalent of murine cathepsin L, is expressed specifically in thymic cortical epithelial cells and generates unique class II MHC binding peptides that are also used in positive selection in the thymic cortex. There is evidence from knockout mice and human polymorphisms in the gene encoding β5t that the generation of unique peptides in the thymic cortex reduces the likelihood of autoreactivity in the pool of positively selected T cells.

The model of positive selection based on weak recognition of self antigens raises a fundamental question: How does positive selection driven by weak recognition of self antigens produce a repertoire of mature T cells specific for foreign antigens? The likely answer is that positive selection allows many different T cell clones to survive, and many of these T cells that recognize self peptides with low affinity will, after maturing, recognize foreign peptides with a high enough affinity to be activated and to generate useful immune responses.

Negative Selection of Thymocytes: Central Tolerance

Thymocytes whose receptors recognize peptide-MHC complexes in the thymus with high avidity undergo apoptosis (called negative selection) or differentiate into regulatory T cells (see Fig. 8.19) **.** Among the double-positive T cells that are generated in the thymus, some may express TCRs that recognize self antigens with high affinity. The peptides present in the thymus are self peptides derived from widely expressed protein antigens and from some proteins thought to be restricted to particular tissues. (Recall that microbes that enter through the common routes, i.e., epithelia, are captured and transported to lymph nodes and tend not to enter the thymus.) In immature T cells, a major consequence of high-avidity antigen recognition is the triggering of apoptosis, leading to death, or deletion, of the cells. Therefore, many of the immature thymocytes that express high-affinity receptors for self antigens in the thymus die, resulting in negative selection of the T cell repertoire. This process eliminates the potentially most dangerous self-reactive T cells and is one of the mechanisms of self tolerance, ensuring that the immune system does not respond to many self antigens. Tolerance induced in immature lymphocytes by recognition of self antigens in the generative (or central) lymphoid organs is also called central tolerance, to be contrasted with peripheral tolerance induced in mature lymphocytes by self antigens in peripheral tissues. We will discuss the mechanisms and physiologic importance of immunologic tolerance in more detail in Chapter 15.

The deletion of immature self-reactive T cells may occur at both the double-positive stage in the cortex and in newly generated single-positive T cells in the medulla. The thymic APCs that mediate negative selection at the double-positive stage are cortical thymic epithelial cells (which also mediate positive selection). Negative selection of single-positive thymocytes may be mediated by bone marrow–derived DCs and macrophages, which are abundant in the medulla, as well as by medullary thymic epithelial cells. Single-positive T cells are drawn to the thymic medulla by chemokines. In the medulla, medullary thymic epithelial cells express a nuclear protein called **AIRE (autoimmune regulator)** that induces low-level expression of many self antigens that are normally expressed only in specific peripheral organs (so-called tissue-restricted antigens). Their AIRE-dependent expression in the thymus makes these tissue-specific antigens available for presentation to immature T cells, facilitating the deletion (negative selection) of these cells. A mutation in the gene that encodes AIRE results in an autoimmune polyendocrine syndrome, underscoring the importance of AIRE in mediating central tolerance to tissue-specific antigens (see Chapter 15).

The mechanism of negative selection in the thymus is the induction of death by apoptosis. Unlike the phenomenon of death by neglect, which occurs in the absence of positive selection, in negative selection, active death-promoting signals are generated when the TCR of immature thymocytes binds with high affinity to antigen. TCR signaling may induce expression of a pro-apoptotic protein called BIM, which probably plays an important role in thymocyte apoptosis during negative selection (see Chapter 15). It is also clear that although high-avidity antigen recognition by immature T cells triggers apoptosis, the same recognition by mature lymphocytes, in concert with other signals, initiates proliferative T cell responses (see Chapter 9). The biochemical basis of this fundamental difference in responses of immature and mature cells is not known.

Recognition of self antigens in the thymus can generate a population of CD4 $^+$ ***regulatory T cells (Treg) that function to prevent autoimmune reactions*** (see Chapter 15) **.** It is not clear which factors determine the choice between the two alternative fates of immature T cells that recognize self antigens with high avidity—namely, the deletion of immature T cells or the development of regulatory T cells. One possibility is that weak signals induce positive selection of thymocytes, strong signals induce negative selection, and intermediate signals induce differentiation into Tregs. But how the level of signals is controlled and how they influence the fate of developing T cells is not clear. Whereas CD28 is not required for the development of naive $CD4^+$ and $CD8^+$ T cells, this costimulatory receptor is required for the generation of some Tregs in the thymus.

γδ T Lymphocytes

TCR αβ- and γδ-expressing thymocytes are separate lineages with a common precursor. In fetal thymuses, the first TCR gene rearrangements involve the γ and δ loci. Recombination of TCR γ and δ loci proceeds in a fashion similar to that of other antigen receptor gene rearrangements, although the order of rearrangement appears to be less rigid than in other loci. In a developing double-negative T cell, rearrangement of TCR β, γ, or δ loci is initially possible. If a cell succeeds in productively rearranging its TCR γ as well as its TCR δ loci before it makes a productive TCR β rearrangement, it is selected into the γδ T cell lineage. This happens in about 10% of developing double-negative T cells. About 90% of the time, a productive TCR β gene rearrangement is made first. In this situation, pre-TCR signaling selects these cells to mature into the αβ T cell lineage, and eventual deletion of TCR δ when TCR α is rearranged (because the TCR δ locus is embedded in the TCR α locus) results in irreversible commitment to the αβ lineage.

The diversity of the γδ T cell repertoire is theoretically even greater than that of the αβ T cell repertoire, in part because the heptamer-nonamer recombination signal sequences adjacent to D segments permit D-to-D joining. Paradoxically, however, the actual diversity of expressed γδ TCRs is limited because only a few of the available V, D, and J segments are used in mature γδ T cells, for unknown reasons. This limited diversity is similar to the limited diversity of the B-1 subset of B lymphocytes and is in keeping with the concept that γδ T cells serve as an early defense against a limited number of commonly encountered microbes at epithelial barriers. The functions of γδ T cells are described in Chapter 10.

Other populations, called NKT cells and MAIT cells, also develop in the thymus; these are described in Chapter 10 as well.

Summary

- B and T lymphocytes arise from a common bone marrow–derived precursor that becomes committed to the lymphocyte lineage. Early maturation is characterized by cell proliferation induced by cytokines, mainly interleukin-7.

- Transcription factors induce the expression of lineage-specific genes and open up specific antigen receptor gene loci.
- The initial expression of pre-antigen receptors and the subsequent expression of antigen receptors are essential for the survival, expansion, and maturation of developing lymphocytes and for selection processes that lead to a diverse repertoire of useful antigen specificities.
- The antigen receptors of B and T cells are encoded by a limited number of gene segments that are spatially segregated in the germline loci but are somatically recombined in developing B and T cells.
- Separate loci encode the immunoglobulin (Ig) heavy chain, Ig κ light chain, Ig λ light chain, T cell receptor (TCR) β chain, TCR α and δ chains, and TCR γ chain. These loci contain V, J, and in the Ig heavy chain and TCR β and δ loci only, D gene segments. Somatic rearrangement of both Ig and TCR loci involves the joining of D and J segments in the loci that contain D segments followed by the joining of the V segment to the recombined DJ segments in these loci, or direct V-to-J joining in the other loci.
- This process of somatic gene recombination is mediated by a recombinase enzyme complex made up of the lymphocyte-specific components RAG-1 and RAG-2.
- The diversity of the antibody and TCR repertoires is generated by the combinatorial associations of multiple germline V, D, and J gene segments and junctional diversity generated by the addition or removal of random nucleotides at the sites of recombination. These mechanisms generate the most diversity at the junctions of the segments that form the third hypervariable regions of both antibody and TCR polypeptides.
- B cell maturation occurs in stages characterized by different patterns of Ig gene rearrangement and expression. In the earliest B cell precursors, called pro-B cells, Ig genes are

initially in the germline configuration, and D to J rearrangement occurs at the Ig heavy chain locus.

- At the pro-B to pre-B cell transition, V-D-J recombination is completed at the Ig H chain locus, and the VDJ exon is spliced to the μ C region exons of the heavy chain RNA to generate a mature mRNA that is translated into the μ heavy chain protein. The pre-B cell receptor is formed by pairing of the μ chain with surrogate light chains and by association with the signaling molecules Igα and Igβ. This receptor delivers survival and proliferation signals and also signals to inhibit rearrangement on the other heavy chain allele (allelic exclusion).
- As cells differentiate into immature B cells, V-J recombination occurs initially at the Ig κ locus, and light chain proteins are expressed. Heavy and light chains are then assembled into intact IgM molecules and expressed on the cell surface. Immature B cells leave the bone marrow to populate peripheral lymphoid tissues, where they complete their maturation. At the mature B cell stage, synthesis of μ and δ heavy chains occurs in parallel mediated by alternative splicing of primary heavy chain RNA transcripts, and membrane IgM and IgD are expressed.
- During B lymphocyte maturation, immature B cells that express high-affinity antigen receptors specific for self antigens present in the bone marrow are induced to edit their receptor genes, or these cells are eliminated.
- T cell maturation in the thymus progresses in stages distinguished by the pattern of expression of the antigen receptor and CD4 and CD8 coreceptor molecules. The earliest T lineage immigrants to the thymus do not express T cell receptors (TCRs) or CD4 or CD8 molecules. The developing thymocytes initially populate the outer cortex, where they undergo proliferation and rearrangement of TCR genes, and express CD3, TCR, CD4, and CD8 molecules.

- At the pre-T stage, thymocytes remain double-negative, but V-D-J recombination is completed at the TCR β chain locus, and TCR β chain polypeptides are produced. The TCR β chain associates with the invariant pre-Tα protein to form a pre-TCR, which transduces signals that inhibit rearrangement on the other β chain allele (allelic exclusion) and promote dual CD4 and CD8 expression. At the $CD4^+CD8^+$ (double-positive) stage, V-J recombination occurs at the TCR α locus, α chain polypeptides are produced, and low levels of TCR are expressed on the cell surface.
- Positive selection of $CD4^+CD8^+$ TCR αβ thymocytes requires low-avidity recognition of peptide–major histocompatibility complex (MHC) complexes. As TCR αβ thymocytes mature, they move into the medulla and become either $CD4^+CD8^-$ or $CD8^+CD4^-$. Lineage commitment accompanying positive selection results in the matching of TCRs that recognize MHC class I with CD8 expression and the silencing of CD4; TCRs that recognize MHC class II molecules are matched with CD4 expression and the loss of CD8 expression.
- Negative selection of $CD4^+CD8^+$ TCR αβ double-positive thymocytes occurs when these cells recognize, with high avidity, antigens that are present in the thymus. This process is responsible for tolerance to many self antigens.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

Early B Cell Development and V(D)J Recombination

Carmona L.M, Schatz D.G. New insights into the evolutionary origins of the recombination-activating gene proteins and V(D)J recombination. *FEBS J* . 2017;284:1590–1605.

Clark M.R, Mandal M, Ochiai K, Singh H. Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signalling. *Nat Rev Immunol* . 2014;14:69–80.

∗Dreyer W.J, Bennett J.C. The molecular basis of antibody formation: a paradox. *Proc Natl Acad Sci U S A* . 1965;54:864–869 *(The hypothesis by Dreyer and Bennett that antibody genes are made up of germline gene segments that need to recombined in B cells paved the way for the eventual studies almost a decade later that established V(D)J recombination as the mechanism by which antibody diversity is generated.).*

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∗Schatz D.G, Oettinger M.A, Baltimore D. The V(D)J recombination activating gene, *RAG-1* . *Cell* . 1989;59:1035–1048 *(This study described the molecular cloning and identification of the enzyme that mediates V(D)J recombination.).*

∗Tonegawa S, Steinberg C, Dube S, Bernardini A. Evidence for somatic generation of antibody diversity. *Proc Natl Acad Sci U S A* . 1974;71:4027–4031 *(This study established that immunoglobulin gene segments exist and are physically separate in non-lymphoid cells but are recombined in B lymphocytes, thus establishing that antibody diversity is generated by gene recombination. This work led to a Nobel prize for Susumu Tonegawa. See https://www.nobelprize.org/prizes/medicine/1987/tonegawa/lecture .).*

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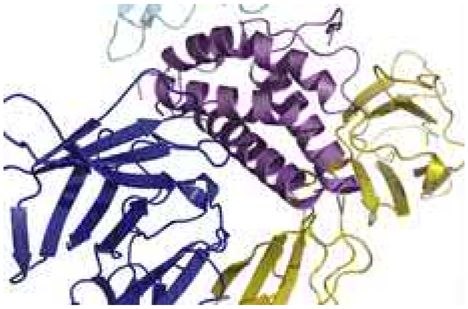
Chapter 9: Activation of T Lymphocytes



- Overview of T Lymphocyte Activation,
- Signals for T Lymphocyte Activation,
    - Recognition of Antigen,
    - Role of Costimulation in T Cell Activation,
- Functional Responses of T Lymphocytes,
    - Changes in Surface Molecules During T Cell Activation,
    - Cytokines in T Cell Activation,
    - Clonal Expansion of T Cells,
    - Differentiation of Activated T Cells Into Effector Cells,
    - Development and Properties of Memory T Cells,
- Decline of T Cell Responses,
- Summary,

The process of T cell activation generates, from a small pool of naive lymphocytes specific for an antigen, a large number of effector cells with the same specificity that function to eliminate that antigen and a population of long-lived memory cells that can rapidly react against the antigen if it is reintroduced. A fundamental characteristic of the T cell response, like all adaptive immune responses, is that it is highly specific for the antigen that elicits the response. Both the initial activation of naive T cells and the effector phases of T cell–mediated adaptive immune responses are triggered by recognition of antigen by the antigen receptors of T lymphocytes. In Chapter 6, we described the specificity of T cells for peptide fragments, derived from protein antigens, which are bound to and displayed by self major histocompatibility complex (MHC) molecules. In Chapter 7, we described the antigen receptors and other molecules that are involved in the activation of T cells by antigens and the biochemical signals initiated by these receptors. In this chapter, we will describe the functional responses of T cells. We begin with a brief overview of T cell activation, discuss the role of costimulators and other signals provided by antigen-presenting cells (APCs) in T cell activation, and introduce the sequence of proliferation and differentiation that occurs when $CD4^+$ and $CD8^+$ T cells recognize foreign antigens. The generation and functions of effector $CD4^+$ and $CD8^+$ T cells are described in more detail in Chapters 10 and 11. Thus, Chapters 9, 10, and 11 together cover the biological functions of T lymphocytes and their roles in cell-mediated immunity.

Overview of T Lymphocyte Activation

In immune responses, T lymphocytes have to recognize the same antigen at two stages: first to initiate the response and later to perform effector functions. Antigen activates naive cells to proliferate and differentiate into effector and memory cells. Then, effector T cells are activated by the same antigen to perform the functions that lead to elimination of the source of the antigen (infected cells or tumors). As we discuss later, the requirements for these two activation events differ in terms of the APCs involved and the other signals needed.

The initial activation of naive T lymphocytes occurs mainly in secondary (peripheral) lymphoid organs, through which these cells normally circulate and where foreign antigens are concentrated and presented by mature dendritic cells (DCs) (Fig. 9.1) **.** Clones of T lymphocytes, each with a different specificity, are generated in the thymus before antigen exposure. Naive T lymphocytes, which have not previously responded to antigens, circulate throughout the body in a resting state, and they acquire powerful functional capabilities only after they are activated. The activation of naive T lymphocytes occurs in specialized regions of lymph nodes, spleen, and mucosal lymphoid tissues, where naive lymphocytes interact with DCs that have captured antigens from tissues or blood (see Chapters 2 and 6).

Antigen recognition together with other activating stimuli induce several biologic responses in T cells: cytokine secretion and increased cytokine receptor expression; proliferation, leading to an increase in the numbers of cells in the antigen-specific clones (called clonal expansion); and differentiation of the naive cells into effector and memory lymphocytes (Fig. 9.2) **.** The process of T cell activation is associated with changes in the expression of numerous surface molecules, some of which are involved in trafficking of T cells and others play important roles in inducing and regulating T cell responses. APCs not only display antigens but also express surface molecules and secrete cytokines that affect the magnitude and nature of the T cell response. The roles of APCs in instructing T cells how to respond to different categories of pathogens are discussed later in this chapter and in Chapter 10.

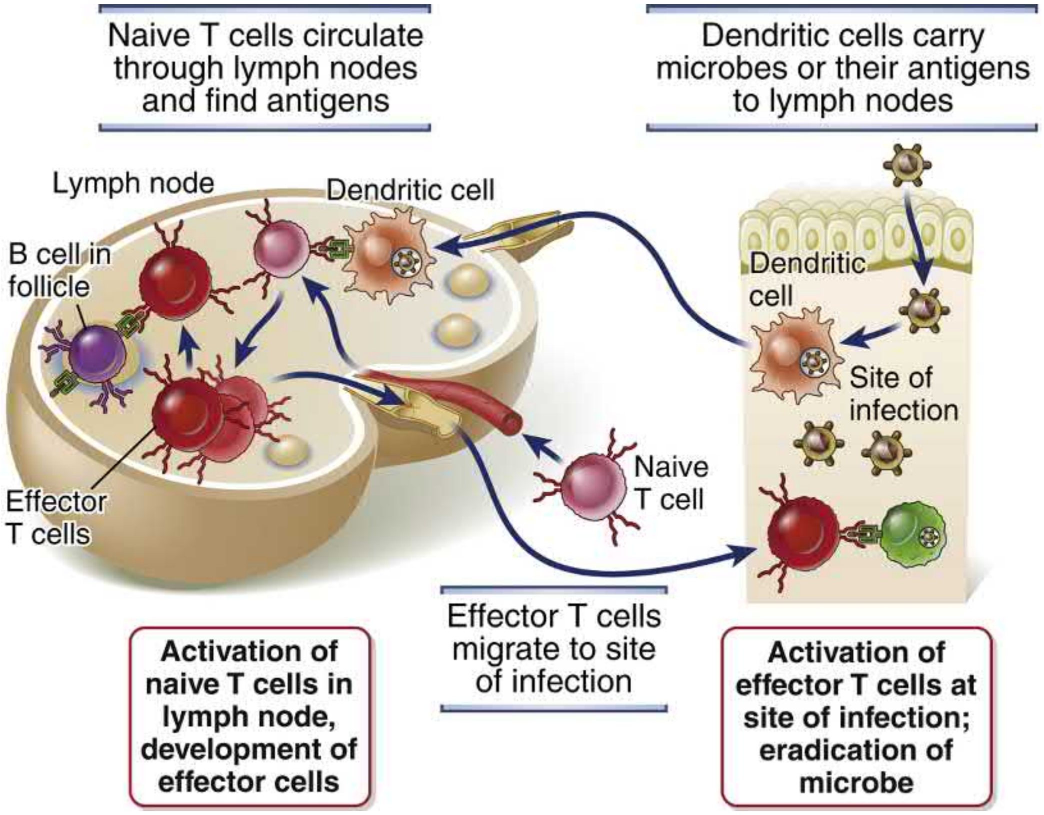

Naive T cells circulate through lymph nodes and find antigens
Dendritic cells carry microbes or their antigens to lymph nodes
Lymph node
Dendritic cell
B cell in follicle
Dendritic cell
Site of infection
Naive T cell
Effector T cells
Effector T cells migrate to site of infection
Activation of naive T cells in lymph node, development of effector cells
Activation of effector T cells at site of infection; eradication of microbe

FIGURE 9.1 Activation of naive and effector T cells by antigen. Antigens that are transported by dendritic cells to lymph nodes are recognized by naive T lymphocytes that recirculate through these lymph nodes. The T cells are activated to differentiate into effector cells, which may remain in the lymphoid organs to help B lymphocytes or migrate to sites of infection, where they are again activated by antigens and perform their various functions, such as macrophage activation.

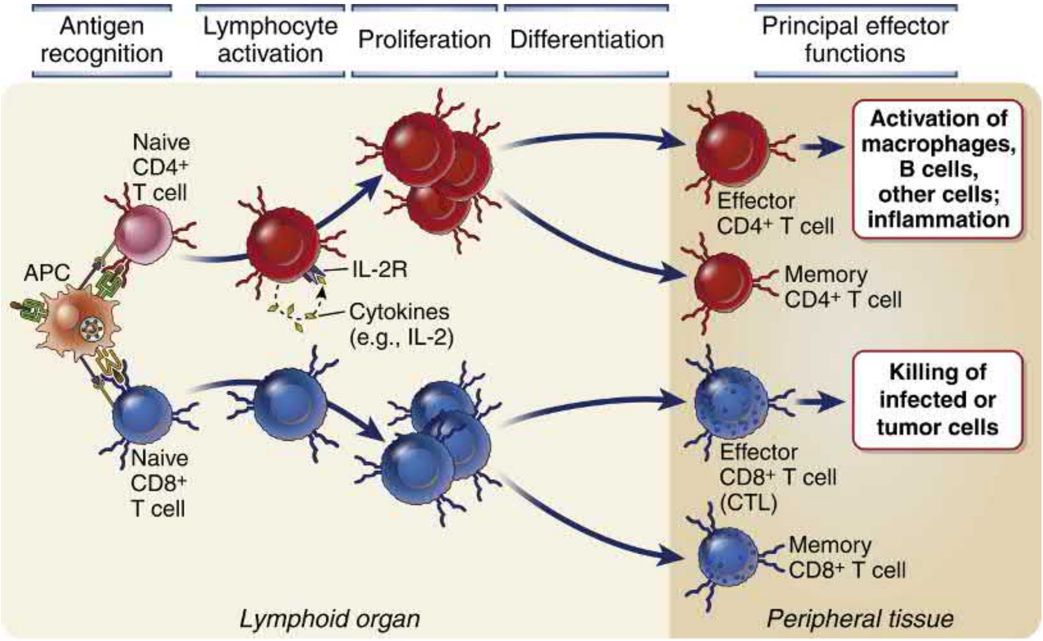

Antigen recognition
Lymphocyte activation
Proliferation
Differentiation
Principal effector functions
Naive CD4+ T cell
APC
IL-2R
Cytokines (e.g., IL-2)
Naive CD8+ T cell
Effector CD4+ T cell
Activation of macrophages, B cells, other cells; inflammation
Memory CD4+ T cell
Killing of infected or tumor cells
Effector CD8+ T cell (CTL)
Memory CD8+ T cell
Lymphoid organ
Peripheral tissue

FIGURE 9.2 Sequence of events in T cell responses.Antigen recognition by T cells induces cytokine (e.g., interleukin-2 *[IL-2]*) secretion, particularly in CD4$^+$ T cells, clonal expansion as a result of cell proliferation, and differentiation of the T cells into effector cells or memory cells. In the effector phase of the response, the effector CD4$^+$ T cells respond to antigen by producing cytokines that have several actions, such as the recruitment and activation of leukocytes and activation of B lymphocytes, while CD8$^+$ CTLs respond by killing other cells and secreting inflammatory cytokines. *APC,* Antigen-presenting cell; *CTL,* cytotoxic T lymphocyte.

Proliferation and differentiation of T cells are regulated by several feedback mechanisms. For example, activated T cells deliver signals back to the APCs, further increasing the APCs' ability to activate T cells in a positive feedback loop. At the same time, some surface molecules expressed on activated T cells as well as cytokines secreted by these cells inhibit further activation, and these negative feedback mechanisms serve to establish safe limits to the response.

Effector T cells recognize antigens in lymphoid organs or in peripheral nonlymphoid tissues and are activated to perform functions that contribute to the elimination of microbes, and, in disease states, induce tissue damage. Whereas naive cells are activated mainly in secondary lymphoid organs, differentiated effector cells may respond to antigens and carry out their functions in any tissue (see Fig. 9.1). The process of differentiation from naive to effector cells gives the cells the capacity to perform specialized functions and the ability to migrate to any site of infection or inflammation. At these sites, the effector cells again encounter the antigen for which they are specific and respond in ways that serve to eliminate the source of the antigen. Effector CD4$^+$ T cells, called helper T cells, can recognize antigens of microbes ingested by macrophages or B cells and activate these cells, and effector CD8$^+$ T cells can recognize antigens on infected or tumor cells and kill these cells. Upon antigen-induced activation, CD4$^+$ T cells secrete cytokines and express cell surface molecules that can activate other immune cells. These effector T cells are classified into subpopulations on the basis of their cytokine profiles and functions (see Chapter 10). Some of the CD4$^+$ effector T cells activate macrophages to kill phagocytosed microbes; others secrete cytokines that recruit different types of leukocytes, such as eosinophils and neutrophils, which destroy different types of pathogens; and yet others remain in lymphoid organs and help B cells differentiate into antibody-secreting plasma cells and memory cells. Cytotoxic T lymphocytes (CTLs), the effector cells of the CD8$^+$ lineage, kill infected cells and tumor cells and also secrete cytokines that activate macrophages and induce inflammation.

Memory T cells that are generated by T cell activation are long-lived and have an enhanced ability to react against the antigen. These cells are present in the recirculating lymphocyte pool and are abundant in mucosal tissues and the skin and in lymphoid organs. After a T cell response wanes, there are many more memory cells of the responding clone than there were naive T cells before the response. These memory cells respond rapidly to subsequent encounters with the antigen and generate new effector cells that can eliminate the antigen.

T cell responses decline after the antigen is eliminated. This process of contraction is important for returning the immune system to a state of equilibrium, or homeostasis. It occurs mainly because the majority of antigen-activated effector T cells die by apoptosis. One reason for this is that as the antigen is eliminated, lymphocytes are deprived of survival stimuli that are normally provided by the antigen and by the costimulators and cytokines produced during inflammatory reactions associated with infections and other types of antigen exposure. In addition, inhibitory mechanisms activated by antigen recognition function to control the magnitude and duration of the response.

With this overview, we will proceed to a discussion of the signals required for T cell activation and the steps in the responses of $CD4^+$ and $CD8^+$ T cells. We will conclude with a discussion of memory cells and the decline of immune responses.

Signals for T Lymphocyte Activation

The proliferation of T lymphocytes and their differentiation into effector and memory cells require antigen recognition, costimulation, and cytokines. In this section, we will summarize the nature of antigens recognized by T cells and discuss specific costimulators and their receptors that contribute to T cell activation. Cytokines are discussed later in this chapter and in Chapter 10.

Recognition of Antigen

Antigen is the necessary first signal for the activation of lymphocytes, ensuring that the resultant immune response is antigen specific. Because $CD4^+$ and $CD8^+$ T lymphocytes recognize peptide-MHC complexes displayed by APCs, they respond to protein antigens, the natural source of peptides, or to chemicals that bind to and modify proteins, thus creating novel peptides. Protein antigens that cross epithelial barriers or are produced in tissues are captured by DCs and transported to lymph nodes. Antigens that enter the circulation may be captured by DCs in the spleen. As discussed in Chapter 6, both naive T cells and mature DCs are drawn to the T cell zones of secondary lymphoid organs by chemokines produced at these sites that engage the CCR7 chemokine receptor on the cells. By the time the mature DCs reach the T cell areas, they display antigenic peptides on MHC molecules and also express costimulators. Some soluble protein antigens in the lymph may be delivered to lymph nodes independent of DCs and are taken up, processed, and presented as peptide-MHC complexes by resident DCs within the lymph nodes. DCs present peptides derived from endocytosed protein antigens mainly in association with class II MHC molecules to naive CD4$^+$ T cells, and peptides derived from cytosolic and nuclear proteins displayed by class I MHC molecules to CD8$^+$ T cells (see Chapter 6).

Naive T lymphocytes move around within secondary lymphoid organs transiently interacting with many DCs and stop when they recognize the antigen for which they express specific receptors. T cells are in constant motion, mainly guided by the fibroblast reticular network, a matrix substratum produced by fibroblastic reticular cells (FRCs) in the T cell zone of the lymphoid organs (see Chapter 2). The DCs in lymphoid organs adhere to the FRC conduits, are relatively immobile, and simultaneously present many different antigens. The T cells move along the conduits, making many successive contacts with different DCs. T cell recognition of antigen being displayed by these DCs results in the generation of biochemical signals that lead to rapid arrest of the T cells. This process stabilizes the contact between the antigen-specific T cells and the relevant antigen-expressing APC and allows the activation program of these T cells to be initiated.

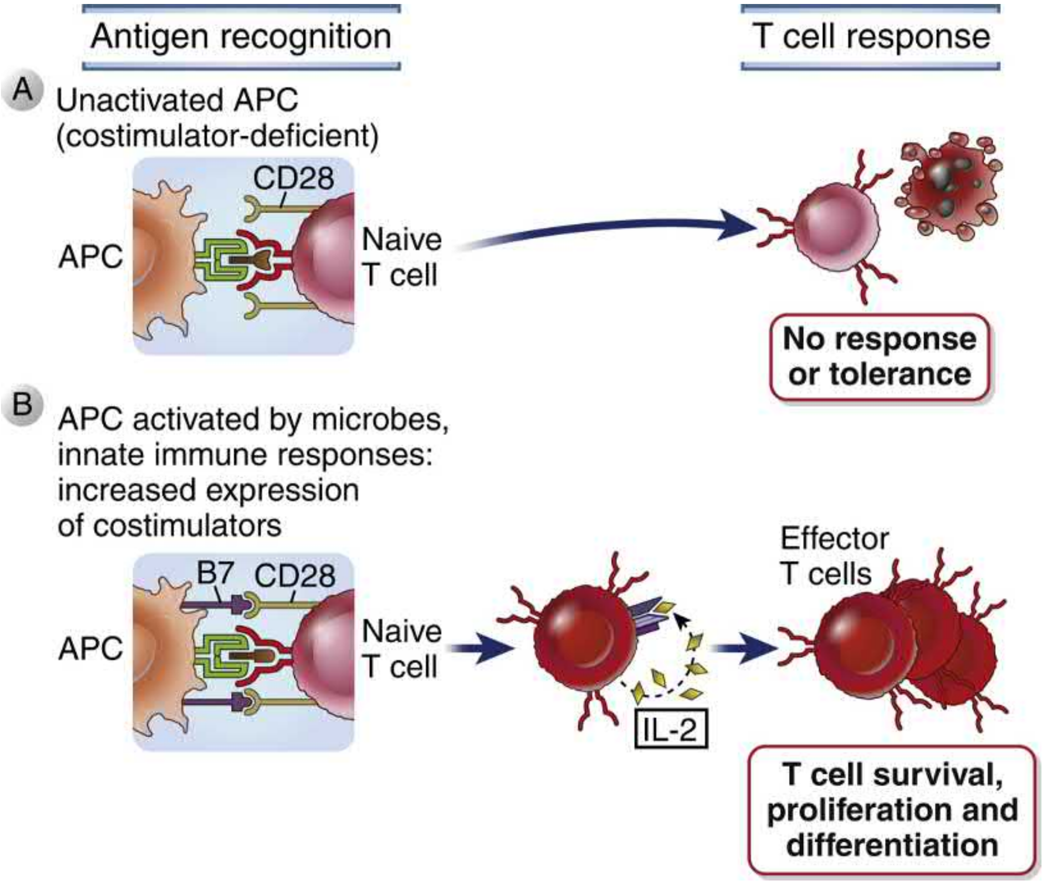

Antigen recognition
T cell response
A
Unactivated APC
(costimulator-deficient)
CD28
APC
Naive
T cell
No response
or tolerance
B
APC activated by microbes,
innate immune responses:
increased expression
of costimulators
B7
CD28
APC
Naive
T cell
IL-2
Effector
T cells
T cell survival,
proliferation and
differentiation

FIGURE 9.3 Functions of costimulators in T cell activation. **A,** The resting antigen-presenting cell *(APC)* (typically dendritic cells presenting self antigens) expresses few or no costimulators and fails to activate naive T cells. Antigen recognition without costimulation may make T cells unresponsive (tolerant) or lead to death of the T cells; we will discuss this phenomenon in Chapter 15. **B,** Microbes and cytokines produced during innate immune responses activate APCs to express costimulators, such as B7 molecules. The APCs (usually presenting microbial antigens) then become capable of activating naive T cells. Activated APCs also produce cytokines such as interleukin-12 *(IL-12),* which stimulate the differentiation of naive T cells into effector cells (not shown).

Differentiated effector T cells can respond to antigens presented by cells other than DCs. In humoral immune responses, B cells present antigens to helper T cells and are the recipients of activating signals from the helper cells (see Chapter 12); in cell-mediated immune responses, macrophages present antigens to and respond to $CD4^+$ T cells (see Chapter 10); and virtually any nucleated cell can present antigens to and be killed by $CD8^+$ CTLs (see Chapter 11).

Role of Costimulation in T Cell Activation

The proliferation and differentiation of naive T cells require signals provided by molecules on APCs, called costimulators, in addition to antigen-induced signals (Fig. 9.3) **.** The requirement for costimulatory signals was first suggested by the experimental finding that T cell antigen receptor signaling alone (e.g., induced by anti-CD3 antibodies that cross-link TCR-CD3 complexes, mimicking antigen) resulted in lower responses than those seen with antigens presented by activated APCs. This result indicated that APCs express molecules that work together with antigen for inducing T cell activation. These molecules were called costimulators, and the second signal for T cell activation was called costimulation, the first signal being antigen. In the absence of costimulation, T cells that encounter antigens fail to respond, enter a state of prolonged unresponsiveness, or die (see Chapter 15).

The B7:CD28 Family of Costimulators

The best characterized costimulatory pathway in T cell activation involves the T cell surface receptor CD28, which binds the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on the surface of activated APCs. CD28 was discovered when stimulatory (agonistic) antibodies against human T cell surface molecules were screened for their ability to enhance T cell responses when added together with an activating anti-CD3 antibody. This was soon followed by the identification of the ligands for CD28, called B7 and later shown to be two homologous proteins, named B7-1 (CD80) and B7-2 (CD86), often collectively called B7. The essential role of CD28 and B7 in T cell activation has been established by the T cell immune deficiency caused by knockout of genes encoding these proteins in mice and by the ability of agents that bind to and block B7 molecules to inhibit T cell responses in experimental animals and in humans. The development of therapeutic agents based on these principles is described later.

B7-1 and B7-2 are structurally similar integral membrane single-chain glycoproteins, each with two extracellular immunoglobulin (Ig)-like domains. CD28 is a disulfide-linked homodimer, each subunit of which has a single extracellular Ig domain. Its cytoplasmic portion contains several tyrosine and proline residues that are involved in binding of adaptor and signaling proteins and in the delivery of activating signals (discussed later). CD28 is expressed on the vast majority of $CD4^+$ T cells, but in adult humans, almost 50% of blood $CD8^+$ T cells, mainly effector and memory cells, express little or no CD28. This loss of CD28 is believed to be the result of chronic antigen stimulation (e.g., in chronic viral infections) and may be a way that T cells limit their own activation by persistent antigens.

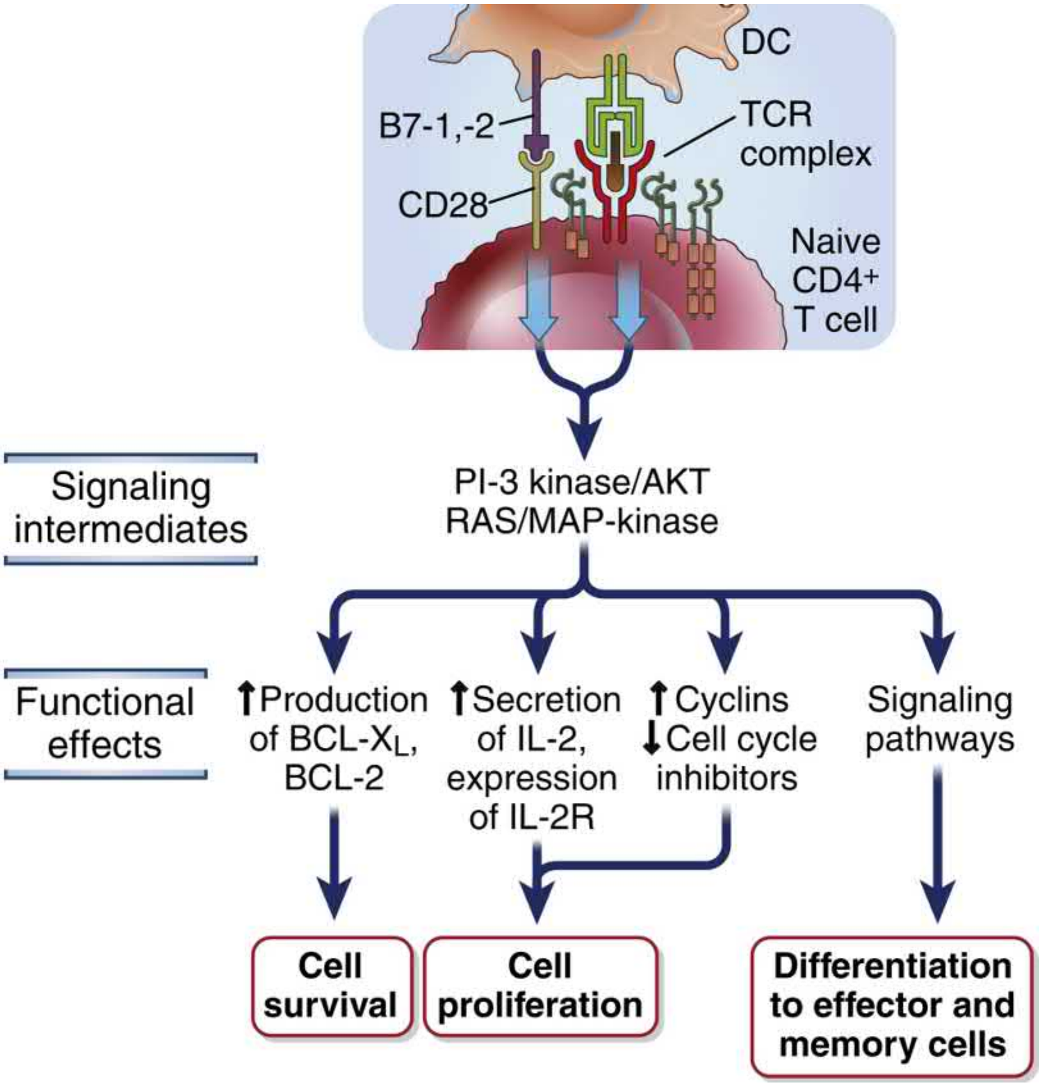

DC
B7-1,-2
TCR complex
CD28
Naive CD4+ T cell
Signaling intermediates
PI-3 kinase/AKT
RAS/MAP-kinase
Functional effects
↑Production of BCL-XL, BCL-2
↑Secretion of IL-2, expression of IL-2R
↑Cyclins
↓Cell cycle inhibitors
Signaling pathways
Cell survival
Cell proliferation
Differentiation to effector and memory cells

FIGURE 9.4 Mechanisms of T cell costimulation by CD28.CD28 engagement by B7 induces several signals, some of which enhance T cell receptor *(TCR)* signals and others that function together with TCR signals to stimulate the expression of survival proteins, cytokines, and cytokine receptors. This results in cell proliferation and differentiation toward effector and memory cells (see Chapters 10 and 11). *IL-2,* Interleukin-2.

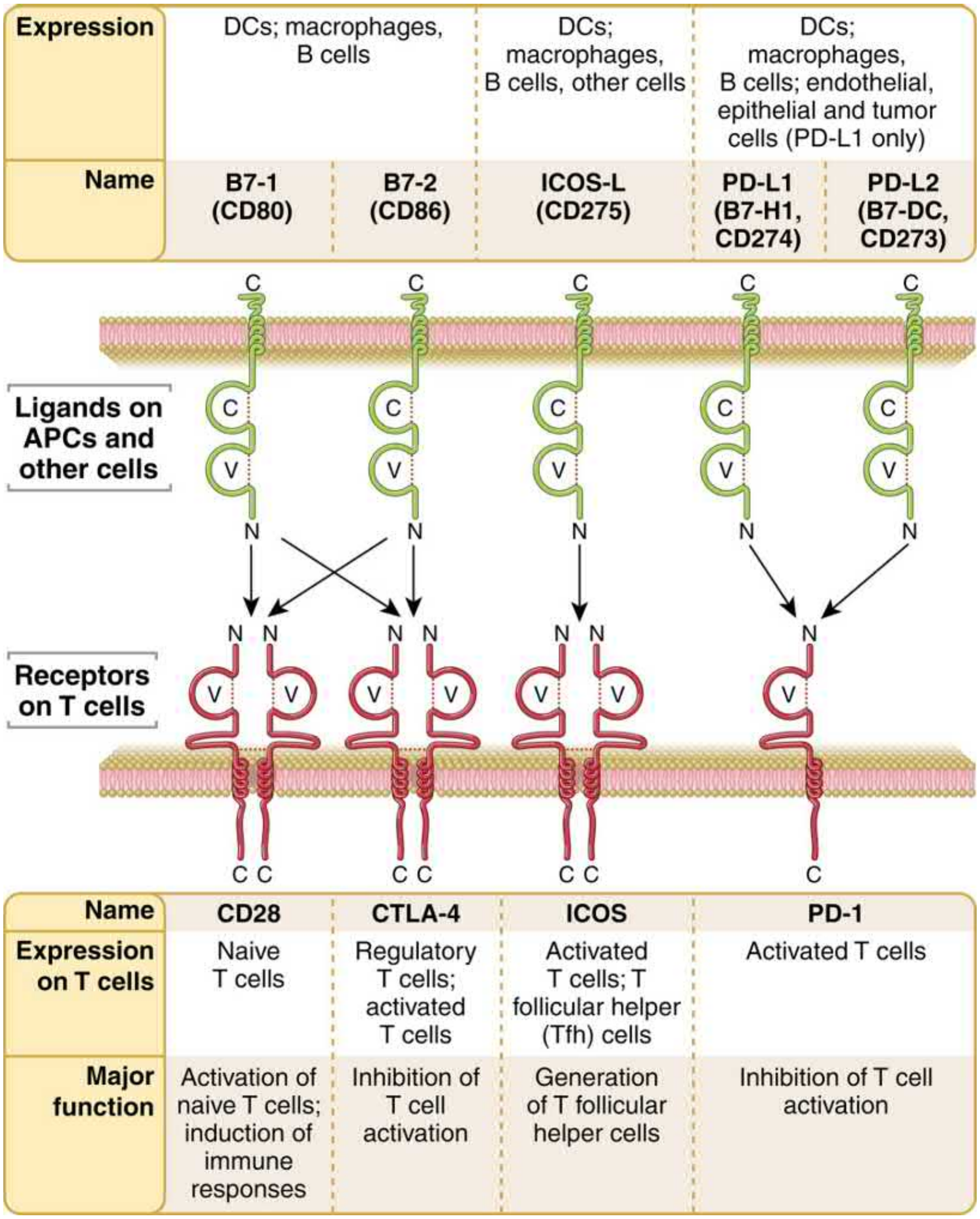
The expression of B7 costimulators is increased by microbial products and the innate immune responses to infections, which ensures that T lymphocytes are activated only when needed. The B7 molecules are expressed mainly on APCs, including DCs, macrophages, and B lymphocytes. They are expressed at low levels on resting APCs and are induced by various stimuli, including microbial products that engage Toll-like receptors and cytokines such as interferon-γ (IFN-γ) produced during innate immune reactions to microbes. The induction of costimulators by microbes and by the cytokines of innate immunity promotes T cell responses to microbial antigens. This illustrates an important role of innate immune responses in enhancing adaptive immunity (see Chapter 4). In addition, activated CD4$^+$ T cells themselves enhance the expression of B7 costimulators on the APCs by a pathway dependent on CD40, described later, and this positive feedback loop serves to amplify T cell responses. Of all potential APCs, mature DCs express the highest levels of costimulators and, as a result, are the most potent stimulators of naive T cells.

In Chapter 6, we mentioned the essential role of **adjuvants** in inducing primary T cell responses to protein antigens such as vaccines. Many adjuvants are products of microbes or mimic molecules produced by microbes and necrotic cells and thus elicit innate immune responses. One of the major functions of adjuvants in T cell activation is to stimulate the expression of B7 costimulators on APCs.

Unactivated, or resting, APCs in normal tissues are capable of presenting self antigens to naive T cells, but because these tissue APCs express only low levels of costimulators, potentially self-reactive T cells that see the self antigens are not activated and may be rendered permanently unresponsive (see Chapter 15). Regulatory T cells, which are important for tolerance to self antigens (see Chapter 15), are also dependent on B7:CD28-mediated costimulation for their generation and maintenance. It is possible that the low levels of B7 costimulators that are constitutively expressed by resting APCs function together with the self antigens that are displayed by these APCs to maintain regulatory T cells.

CD28 signals work in cooperation with antigen recognition to promote the survival, proliferation, and differentiation of the

antigen-specific T cells. Costimulatory signaling via CD28 amplifies signaling pathways that are also induced downstream of the TCR (see Chapter 7) and may trigger additional signals that cooperate with TCR-induced signals (Fig. 9.4). PI3-kinase is recruited to the cytoplasmic tail of CD28, and this in turn activates the kinase AKT, which alters cellular metabolism and promotes cell survival. CD28 can also contribute to the activation of the JNK mitogen-activated protein (MAP) kinase via the RAC small G protein and can amplify the activation of nuclear factor κB (NF-κB). The net results of these signaling pathways in T cells are the increased expression of anti-apoptotic proteins such as BCL-2 and BCL-X_L that promote cell survival; increased metabolic activity; enhanced proliferation; production of cytokines such as IL-2; and differentiation of the naive T cells into effector and memory cells.



Name	B7-1 (CD80)	B7-2 (CD86)	ICOS-L (CD275)	PD-L1 (B7-H1, CD274)	PD-L2 (B7-DC, CD273)
Expression	DCs; macrophages, B cells		DCs; macrophages, B cells, other cells	DCs; macrophages, B cells; endothelial, epithelial and tumor cells (PD-L1 only)	

Name	CD28	CTLA-4	ICOS	PD-1
Expression on T cells	Naive T cells	Regulatory T cells; activated T cells	Activated T cells; T follicular helper (Tfh) cells	Activated T cells
Major function	Activation of naive T cells; induction of immune responses	Inhibition of T cell activation	Generation of T follicular helper cells	Inhibition of T cell activation

FIGURE 9.5 The major members of the B7 and CD28 families. The known B7 family ligands are expressed on antigen-presenting cells *(APCs)* (dendritic cells *[DCs],* macrophages, and B cells), and CD28 family receptors are expressed mainly on T cells. Different CD28 family members stimulate or inhibit different stages and types of T cell responses. The functions of cytotoxic T

lymphocyte antigen 4 *(CTLA-4)* and programmed cell death protein-1 *(PD-1)* are discussed in Chapter 15, and the role of inducible costimulator *(ICOS)* in the generation and function of T follicular helper cells is discussed in Chapter 12. Other widely distributed molecules with limited homology to B7, such as B7-H3 and B7-H4, have been identified, but their physiologic roles are not yet established. Other inhibitory receptors have also been identified, such as BTLA, TIM-3, and TIGIT, but these are not homologous to CD28 and are not shown.

Previously activated effector and memory T cells are less dependent on costimulation by the B7:CD28 pathway than are naive cells. This property of effector and memory cells enables them to respond to antigens presented by various APCs that may reside in nonlymphoid tissues and may express no or low levels of B7. For instance, the differentiation of $CD8^+$ T cells into effector CTLs requires costimulation, but effector CTLs can respond to and kill other cells that do not express costimulators.

Other receptors homologous to CD28 and their ligands homologous to B7 have been identified, and these proteins regulate T cell responses both positively and negatively (Fig. 9.5) **.** Following the demonstration of the importance of B7 and CD28, several other proteins structurally related to B7-1 and B7-2 or to CD28 were discovered. Some members of the B7 and CD28 families are involved in T cell activation, and others are inhibitors of T cells. In addition to CD28, the costimulator whose function is best understood is ICOS (inducible costimulator, CD278). Its ligand, called ICOS-L (CD275), is expressed on DCs, B cells, and other cell populations. ICOS plays an essential role in T cell–dependent antibody responses, particularly in the germinal center reaction. It is required for the development and activation of T follicular helper cells, which are essential for the formation of germinal centers and for the generation of B cells that produce high-affinity antibodies (see Chapter 12).

The outcome of T cell activation is influenced by a balance between engagement of activating and inhibitory receptors of the CD28 family. The inhibitory receptors of the CD28 family are **CTLA-4** (cytotoxic T lymphocyte antigen 4, CD152) and **PD-1** (programmed cell death protein 1, CD279). (Their names do not accurately reflect their distribution or function.) The function of these receptors is often described as **coinhibtion**, to contrast with CD28 and ICOS whose function is costimulation. (Note that sometimes, CD28, the activating receptor, is called a costimulator.) Both inhibitory receptors are expressed following T cell activation and function to limit immune responses. The concept that a balance between activating and inhibitory receptors controls the magnitude of responses in the immune system was discussed in Chapter 4 in the context of natural killer (NK) cells (see Fig. 4.10). A similar idea is applicable to responses of T and B lymphocytes, although the receptors involved are quite different. Because the physiologic function of the inhibitory receptors CTLA-4 and PD-1 is to prevent and control responses to self and microbial antigens and deficiencies in their functions due to gene mutations or blockade with antibodies cause autoimmune diseases, we will discuss them in more detail in Chapter 15, when we consider immunologic tolerance and autoimmunity. Suffice it to say here that CTLA-4 functions as a competitive inhibitor of CD28 by binding more strongly to B7 molecules, and PD-1 recruits and activates a tyrosine phosphatase that blocks tyrosine kinase–dependent signaling by the TCR and CD28.

It is likely that the various costimulators and inhibitory receptors of the B7-CD28 family serve distinct roles in different immune responses or at different stages of a response. It is thought that the CD28:B7 interaction is most important for initiating T cell responses by activating naive T cells; ICOS:ICOS-ligand interactions are critical for helper T cell–dependent antibody responses; CTLA-4:B7 interactions inhibit the initial activation of T lymphocytes in secondary lymphoid organs; and PD1:PD-ligand interactions mainly inhibit the activation of effector T cell responses to antigens, such as CD8$^+$ T cell responses, especially in nonlymphoid tissues.

Other Costimulatory Pathways

Many other T cell surface molecules have been shown to deliver costimulatory signals in vitro, but their physiologic role in promoting T cell activation is less clear than that of the CD28 family. Several putative costimulatory receptors belong to the large tumor necrosis factor receptor (TNFR) superfamily, and their ligands are members of the TNF family. Many of these receptors are expressed on activated T cells and regulatory T cells and have been shown to stimulate or to inhibit immune responses under various experimental conditions. OX40 (CD134) is a TNFR family member, expressed on activated CD4$^+$ and CD8$^+$ T cells, that functions to maintain cell survival and sustained responses. Its ligand, OX40L, is expressed on activated APCs. 4-1BB (CD137) and CD27 are two other TNFR superfamily molecules that are expressed on activated and memory T cells as well as regulatory T cells; their roles in regulating immune responses are not well defined. T cells also express numerous inhibitory receptors in addition to CTLA-4 and PD-1, but their physiologic functions are also not well established (see Chapter 15). Two that have received considerable interest are TIM-3 and LAG-3. Inherited mutations in TIM-3 are associated with systemic inflammatory disease.

The interaction of CD40L on T cells with CD40 on APCs enhances T cell responses by activating the APCs. CD40 ligand (CD40L) is a TNF superfamily membrane protein that is expressed primarily on activated T cells, and CD40 is a member of the TNFR superfamily expressed on B cells, macrophages, and DCs. The functions of CD40 in activating macrophages in cell-mediated immunity and activating B cells in humoral immune responses are described in Chapters 10 and 12, respectively. Activated helper T cells express CD40L, which engages CD40 on the APCs and activates the APCs, making them more potent by enhancing their expression of B7 molecules and cytokines such as IL-12 that promote T cell differentiation (Fig. 9.6). This phenomenon is sometimes called licensing, meaning that activated T cells license APCs to become more powerful stimulators of immune responses. Thus, the CD40 pathway indirectly amplifies T cell responses by inducing costimulators on APCs, but CD40L does not by itself function as a costimulator for T cells.

Therapeutic Targeting of Costimulators

Based on the understanding of costimulatory pathways, therapeutic agents have been developed for controlling injurious immune responses by inhibiting costimulation, called **costimulatory blockade** (Fig. 9.7). CTLA-4-Ig, a fusion protein consisting of the extracellular domain of CTLA-4 and the Fc portion of human IgG, binds to B7-1 and B7-2 and blocks the B7:CD28 interaction. The reason for the use of the extracellular domain of CTLA-4 rather than of CD28 to bind to and block B7 molecules is that CTLA-4 has a higher affinity for B7 than does CD28. Attachment of the Fc portion of IgG increases the in vivo half-life of the protein (see Chapter 5). CTLA-4-Ig is an approved therapy for rheumatoid arthritis and transplant rejection. Inhibitors of the CD40L:CD40 pathway are in clinical trials for transplant rejection and autoimmune diseases.

Antibodies that block the CTLA-4 and PD-1 inhibitory receptors are approved for the immunotherapy of tumors; they work by preventing CTLA-4 or PD-1 from binding their ligands, thereby reducing inhibition and thus enhancing T cell activation and enabling the cancer-bearing individual to mount more effective antitumor immune responses (see Chapter 18). Because these inhibitory receptors impose checkpoints on immune responses, blocking them therapeutically to enhance immune responses is called **checkpoint blockade.** As one might predict from the role of these inhibitory receptors in maintaining self-tolerance, blocking them for cancer immunotherapy induces autoimmune reactions in many patients.

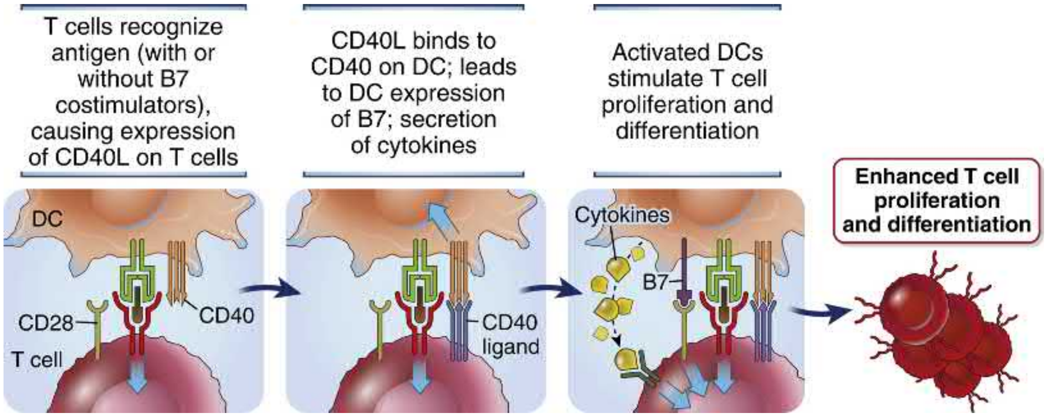

T cells recognize antigen (with or without B7 costimulators), causing expression of CD40L on T cells
CD40L binds to CD40 on DC; leads to DC expression of B7; secretion of cytokines
Activated DCs stimulate T cell proliferation and differentiation
Enhanced T cell proliferation and differentiation
DC
CD28
CD40
T cell
CD40 ligand
Cytokines
B7

FIGURE 9.6 Role of CD40 in T cell activation.Antigen recognition by T cells induces the expression of CD40 ligand *(CD40L)* on the activated T cells. CD40L engages CD40 on APCs and may stimulate the expression of more B7 molecules and the secretion of cytokines that activate T cells. Thus, CD40L on the T cells makes the APCs better at promoting and amplifying T cell activation. *APCs,* Antigen-presenting cells; *DCs,* dendritic cells.

Functional Responses of T Lymphocytes

The earliest responses of antigen-stimulated T cells consist of changes in the expression of various surface molecules, including cytokine receptors, as well as the secretion of cytokines. These are followed by proliferation of the antigen-specific cells, driven in part by the secreted cytokines, and then by differentiation of the activated cells into effector and memory cells. In the remainder of this chapter, we will describe these steps, their underlying mechanisms, and their functional consequences.

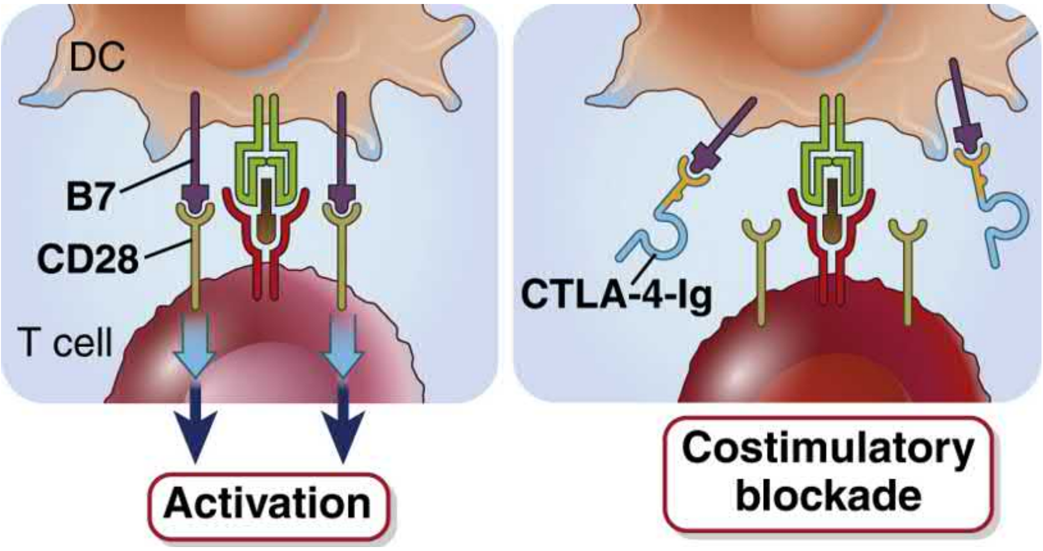


FIGURE 9.7 The mechanism of therapeutic costimulatory blockade. **A,** The normal T cell response induced by antigen recognition and costimulation mediated by B7-CD28. **B,** A fusion protein consisting of the extracellular portion of cytotoxic T lymphocyte antigen 4 *(CTLA-4)* and the Fc tail of an immunoglobulin G (IgG) molecule is used to bind to and block B7 molecules, thus preventing their interaction with the activating receptor CD28 and inhibiting T cell activation. *DC,* Dendritic cell.

Changes in Surface Molecules During T Cell Activation

After activation by antigen recognition and costimulation, there are characteristic changes in the expression of various surface molecules in T cells (Fig. 9.8) **.** Many of the molecules that are expressed in activated T cells are also involved in the functional responses of the cells.

- ***CD69.*** Within a few hours, T cells increase their expression of CD69. This protein binds to and reduces surface expression of the sphingosine-1 phosphate receptor 1 (S1PR1), which we described in Chapter 3 as a receptor that mediates egress of T cells from secondary lymphoid organs. The consequence of decreased S1PR1 expression is that activated T cells are retained in lymphoid organs long enough to receive the signals that initiate their proliferation and differentiation into effector and memory cells. After that occurs, CD69 expression decreases, the activated T cells reexpress high levels of S1PR1, and therefore the effector and memory cells can exit the lymphoid organs (see Chapter 3).

- ***CD25 (IL-2Rα).*** The expression of this component of the receptor for the growth factor interleukin-2 (IL-2) enables activated T cells to respond to this cytokine. This process is described later.
- ***CD40 ligand (CD40L, CD154).*** Within 24 to 48 hours after antigen recognition, $CD4^+$ T cells express high levels of the ligand for CD40. The expression of CD40L enables these activated T cells to mediate their key effector functions, which are to help macrophages and B cells. In addition, as discussed earlier, CD40L on the T cells activates DCs to become better APCs, thus providing a positive feedback mechanism for amplifying T cell responses.
- ***CTLA-4 and PD-1.*** CTLA-4 is expressed on T cells within 24 to 48 hours after antigen recognition, and PD-1 may be induced even more rapidly. The role of these inhibitors in limiting immune responses is described later in this chapter, in self tolerance in Chapter 15, and in tumor immunity in Chapter 18.

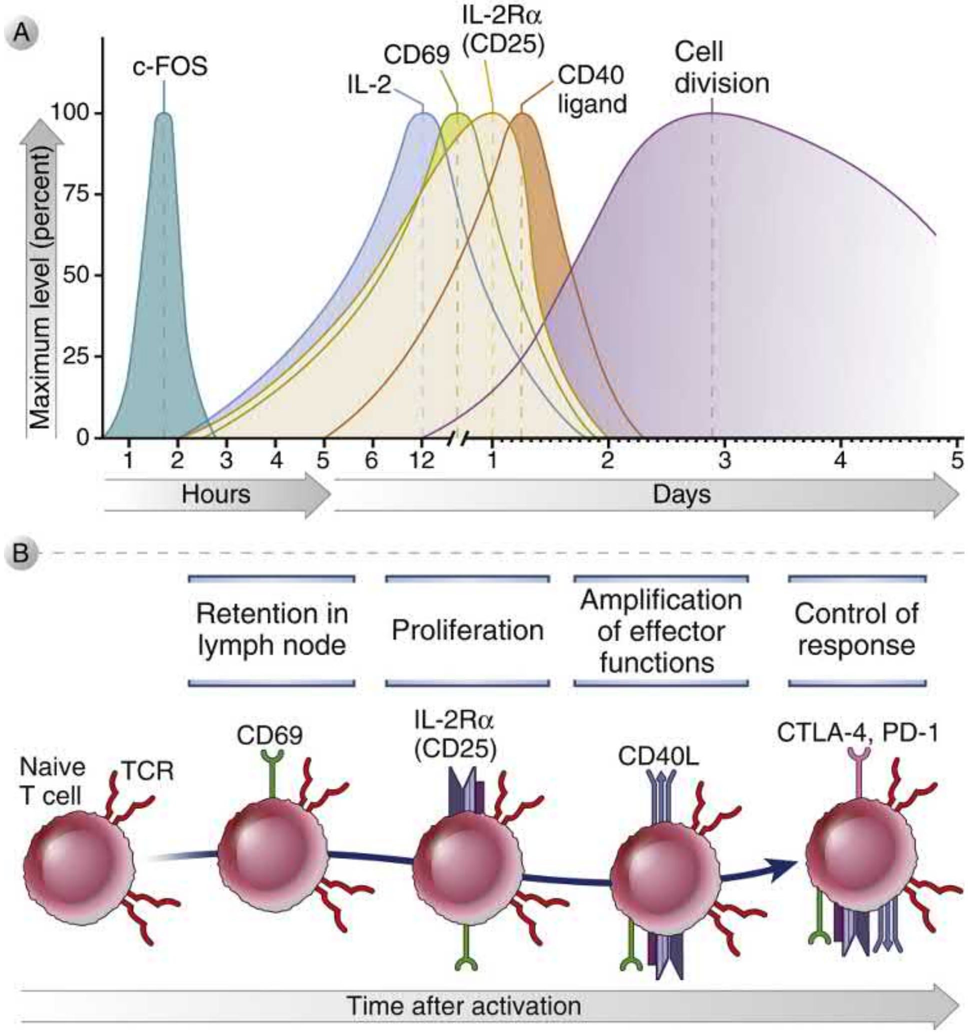


FIGURE 9.8 Changes in surface molecules after T cell activation. **A,** The approximate kinetics of expression of selected molecules during activation of T cells by antigens and costimulators are shown. The illustrative examples include a transcription factor (c-FOS), a cytokine *(IL-2),* and surface proteins. These proteins are typically expressed at low levels in naive T cells and are induced by activating signals. CTLA-4 (cytotoxic T lymphocyte antigen 4) and PD-1 (programmed cell death protein 1) are induced hours or 1 to 2 days after initial activation. The kinetics are estimates and will

vary with the nature of the antigen, its dose and persistence, and the type of adjuvant. **B,** The major functions of selected surface molecules are shown and described in the text. *CD40L,* CD40 ligand; *IL-2R*, interleukin-2 receptor.

- ***Adhesion molecules and chemokine receptors.*** During activation, T cells reduce expression of molecules that bring them to secondary lymphoid organs (such as L-selectin [CD62L] and the chemokine receptor CCR7) and increase the expression of molecules that are involved in their migration to peripheral sites of infection and tissue injury (such as the integrins LFA-1 and VLA-4, the ligands for E- and P-selectins, and various chemokine receptors). These molecules and their roles in T cell migration were described in Chapter 3. Activation also increases the expression of CD44, a receptor for the extracellular matrix molecule hyaluronan. Binding of CD44 to its ligand helps retain effector T cells in the tissues at sites of infection and tissue damage.

Cytokines in T Cell Activation

Numerous cytokines play critical roles in adaptive immune responses. $CD4^+$ helper T cells make the largest amount and variety of these cytokines, but some are also produced by $CD8^+$ T cells and APCs. Cytokines secreted by DCs and other APCs are especially important for the differentiation of naive T cells into different types of effector cells. Various cytokines are involved in the proliferation and differentiation of antigen-stimulated T cells and in the effector functions of these cells. Most of these cytokines act on the cells that produce them (autocrine action) or on nearby cells (paracrine action).

The roles of cytokines in the effector functions of T cells are described in Chapters 10 and 11. Here we discuss IL-2, the prototype of a T cell–derived cytokine that stimulates T cell responses.

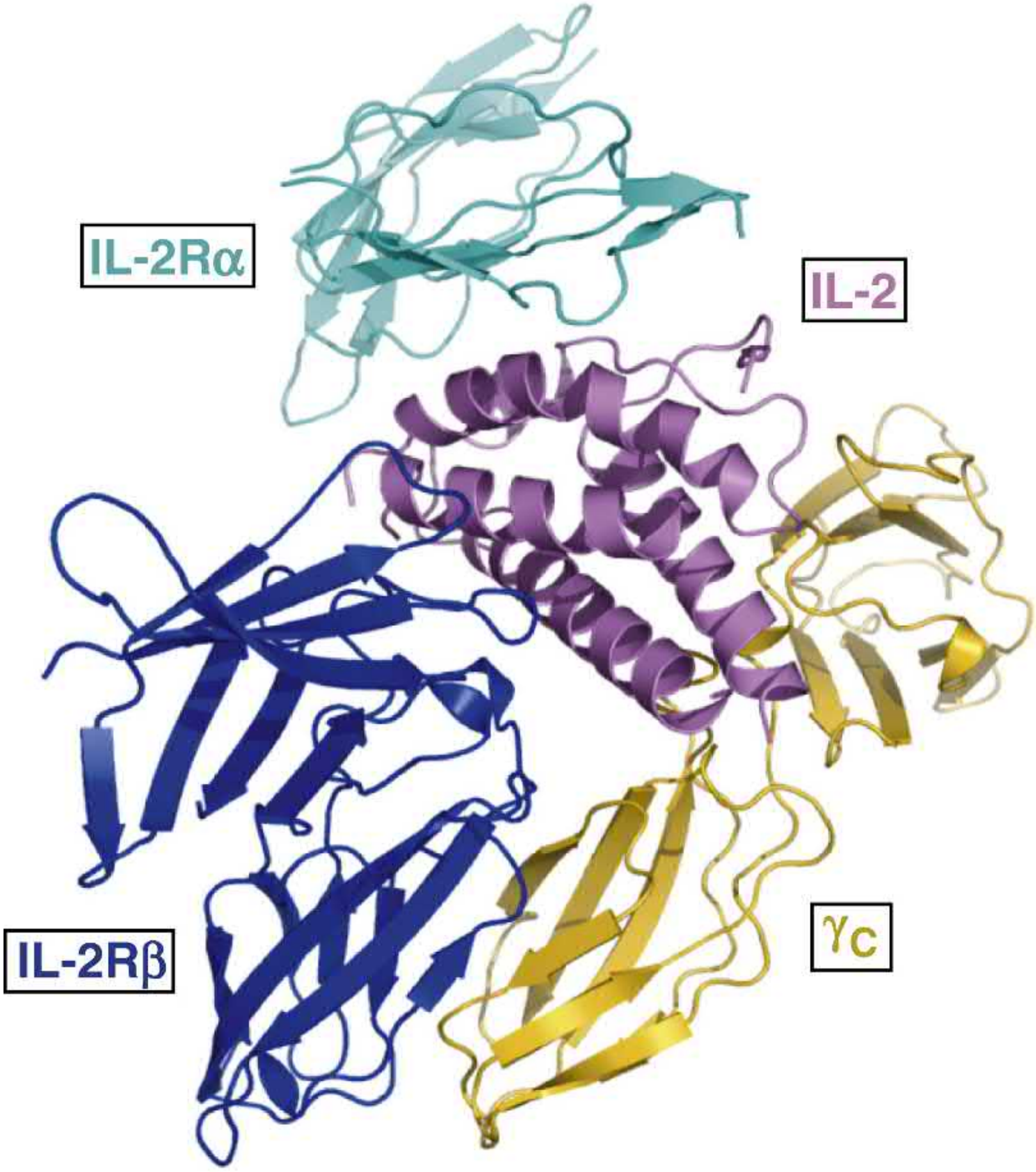


FIGURE 9.9 Structure of interleukin-2 and its receptor. The crystal structure of interleukin-2 *(IL-2)* and its trimeric receptor shows how the cytokine interacts with the three chains of the receptor.

From Wang X, Rickert M, Garcia KC. Structure of the quaternary complex of interleukin-2 with its α, β, and γc receptors, *Science* 310:1159–1163, 2005, with the permission of the publishers. Courtesy Drs. Patrick Lupardus and K. Christopher Garcia, Stanford University School of Medicine, Palo Alto, California.

IL-2 Secretion and IL-2 Receptor Expression

IL-2 is a growth, survival, and differentiation factor for T lymphocytes that plays a major role in the proliferation of antigen-stimulated T cells and in the maintenance of functional regulatory T cells. IL-2 acts on the same cells that produce it and on adjacent cells (i.e., it functions as an autocrine and paracrine cytokine).

IL-2 is produced mainly by CD4$^+$ T lymphocytes rapidly after recognition of antigen and costimulators. Activation of T cells stimulates transcription of the *IL2* gene and synthesis and secretion of the protein. IL-2 production is rapid and transient, starting within 1 to 2 hours after antigen recognition, peaking at about 8 to 12 hours, and declining by 24 hours. Secreted IL-2 is a 14- to 17-kD globular glycoprotein containing four α helices (Fig. 9.9). It is the prototype of the four-α-helical cytokine family that interacts with type I cytokine receptors (see Chapter 7).

The high-affinity IL-2 receptor (IL-2R) is transiently expressed on activation of naive and effector T cells; regulatory T cells always express this receptor. The IL-2R consists of three noncovalently associated proteins, IL-2Rα (CD25), IL-2/15Rβ (CD122), and γc (CD132). Of the three chains, only IL-2Rα is unique to the IL-2R. The β chain is also part of the IL-15 receptor. The γ chain is shared with a number of cytokine receptors, including those for IL-4, IL-7, IL-9, IL-15, and IL-21, and is therefore called the common γ chain (γc). Both the β and γc chains engage JAK (Janus kinase)-STAT (signal transducers and activators of transcription) signaling pathways (see Chapter 7). IL-2R$\beta\gamma$c complexes are expressed at low levels on resting T cells (and on NK cells) and bind IL-2 with an equilibrium dissociation constant (K_d) of approximately 10^{-9} M (Fig. 9.10). Expression of IL-2Rα and, to a lesser extent, of IL-2Rβ is increased on activation of naive CD4$^+$ and CD8$^+$ T cells. The α chain associates with the $\beta\gamma$c complex to form the complete IL-2R, the IL-2R$\alpha\beta\gamma$c complex, which can bind IL-2 more tightly, with a K_d of approximately 10^{-11} M. Growth stimulation of activated T cells occurs at a similarly low IL-2 concentration. Because both IL-2 secretion and IL-2Rα production occur in response to antigen stimulation, the antigen-activated T cells are the ones that proliferate preferentially in response to the cytokine, compared with bystander cells that have not recognized antigen. IL-2, produced in response to antigen stimulation, is itself a stimulus for induction of IL-2Rα, providing a feedback mechanism by which T cell responses amplify themselves. Activated T cells express IL-2Rα transiently, long enough to respond to the growth factor and proliferate. CD4$^+$ regulatory T cells express the complete IL-2R complex and require IL-2 for their maintenance, as discussed in the following section and in Chapter 15. Chronic T cell stimulation leads to shedding of IL-2Rα, and an increased level of shed IL-2Rα in the serum is a marker of strong antigenic stimulation (e.g., acute rejection of a transplanted organ).

Functions of IL-2

The biology of IL-2 is fascinating because it plays critical roles in both promoting and controlling T cell responses and functions (Fig. 9.11).

- ***IL-2 stimulates the survival, proliferation, and differentiation of antigen-activated T cells.*** IL-2 promotes survival of cells by inducing the anti-apoptotic protein BCL-2. It stimulates cell cycle progression through activation of mTOR (mechanistic target of rapamycin) (see Chapter 7), which induces the synthesis of cyclins and relieves a block in cell cycle progression through degradation of the cell cycle inhibitor p27. In addition, IL-2 increases production of effector cytokines, such as IFN-γ and IL-4, by T cells.
- ***IL-2 is required for the survival and function of regulatory T cells,*** which suppress immune responses against self and other antigens. These cells constitutively express the complete IL-2 receptor, including the α chain CD25, and are much more sensitive to IL-2 than are activated and effector T cells. We will discuss this role of IL-2 in more detail in

Chapter 15, when we describe the properties and functions of regulatory T cells. An interesting feature of this function of IL-2 is that regulatory T cells do not produce the cytokine, so they depend on IL-2 made by other T cells responding to antigens (see Fig. 9.11B).

Clonal Expansion of T Cells

T cell proliferation in response to antigen recognition is mediated by a combination of signals from the antigen receptor, costimulators, and autocrine growth factors, primarily IL-2. The expansion of antigen-specific clones that results from this proliferation converts the small pool of naive antigen-specific lymphocytes into the large number of cells required to eliminate the antigen. Before antigen exposure, the frequency of naive T cells specific for any antigen is 1 in 10^5 to 10^6 lymphocytes or fewer. After microbial antigen exposure, the frequency of $CD8^+$ T cells specific for that microbe may increase to as many as 1 in 3 $CD8^+$ T lymphocytes, representing a greater than 50,000-fold expansion of antigen-specific $CD8^+$ T cells, and the number of specific $CD4^+$ cells increases up to 1 in 100 $CD4^+$ lymphocytes, or a 1000-fold expansion (Fig. 9.12). Studies in mice first showed this tremendous expansion of the antigen-specific population in some acute viral infections, and remarkably it occurred within as little as 1 week after infection. Equally remarkable was the finding that during this massive expansion of antigen-specific clones, bystander T cells not specific for the virus did not proliferate. The expansion of T cells specific for the Epstein-Barr virus and human immunodeficiency virus in acutely infected humans is also on this order of magnitude.

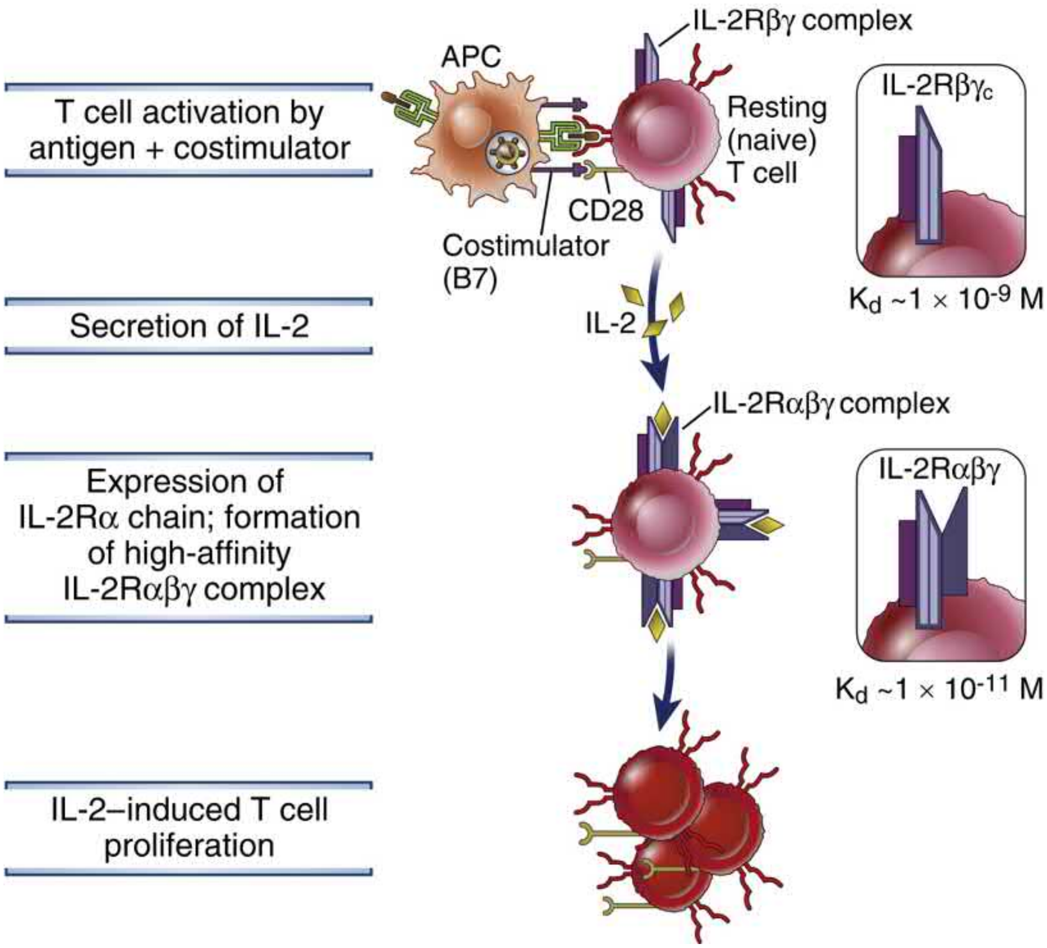

IL-2Rβγ complex
APC
T cell activation by antigen + costimulator
Resting (naive) T cell
IL-2Rβγc
CD28
Costimulator (B7)
Kd ~1 × 10-9 M
Secretion of IL-2
IL-2
IL-2Rαβγ complex
IL-2Rαβγ
Expression of IL-2Rα chain; formation of high-affinity IL-2Rαβγ complex
Kd ~1 × 10-11 M
IL-2–induced T cell proliferation

FIGURE 9.10 Regulation of interleukin-2 receptor expression.Resting (naive) T lymphocytes express the IL-2Rβγc complex, which has a moderate affinity for IL-2. Activation of the T cells by antigen, costimulators, and IL-2 itself leads to expression of the IL-2Rα chain (also called CD25) and increased levels of the high-affinity IL-2Rαβγc complex. *IL-2,* Interleukin-2.

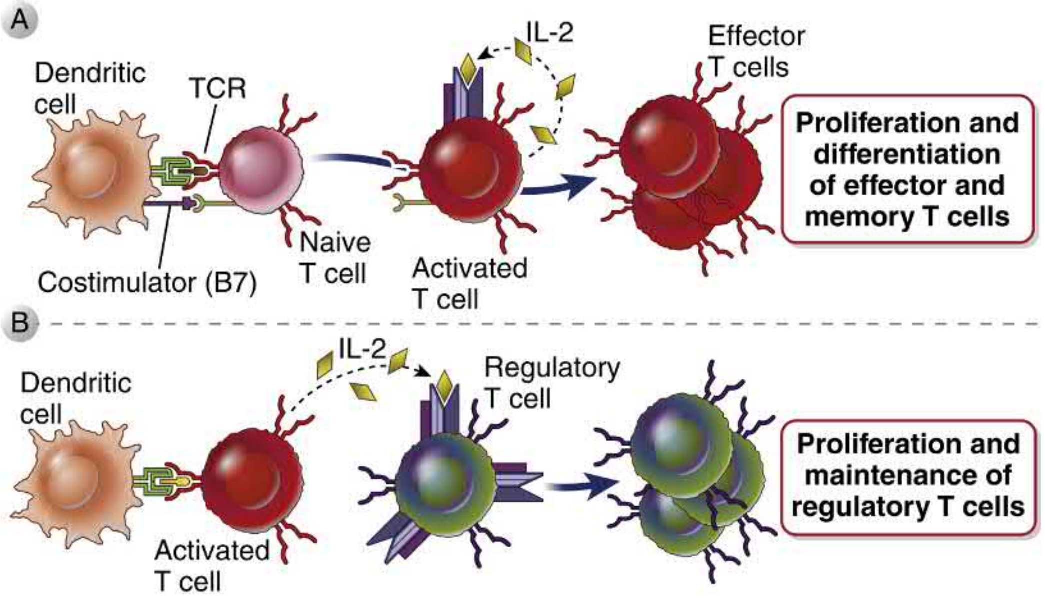

A
Dendritic cell
TCR
IL-2
Effector T cells
Proliferation and differentiation of effector and memory T cells
Costimulator (B7)
Naive T cell
Activated T cell
B
IL-2
Regulatory T cell
Dendritic cell
Proliferation and maintenance of regulatory T cells
Activated T cell

FIGURE 9.11 Biologic actions of interleukin-2. **A,** Interleukin-2 *(IL-2)* stimulates the survival, proliferation, and differentiation of T lymphocytes, acting as an autocrine growth factor, leading to the generation of effector and memory cells. **B,** IL-2 also promotes the survival of regulatory T cells and maintains their functional capability, and thus controls immune responses (e.g., against self antigens). *TCR,* T cell receptor.

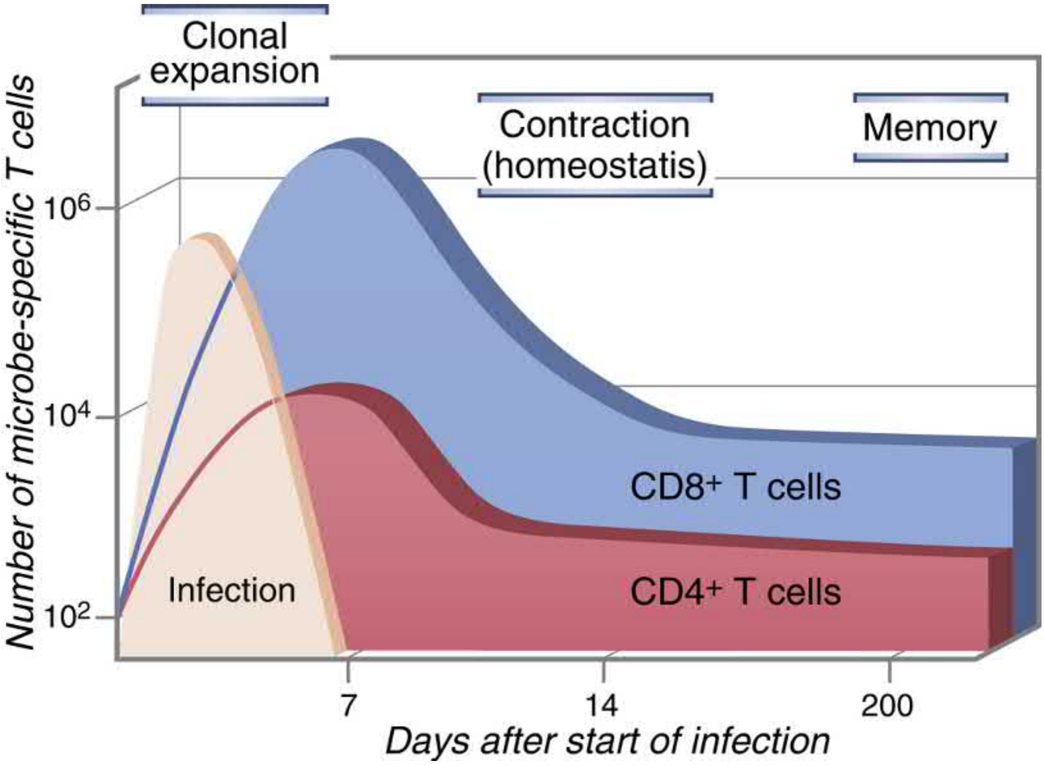


FIGURE 9.12 Clonal expansion of T cells. The numbers of CD4$^+$ and CD8$^+$ T cells specific for microbial antigens and the expansion and decline of the cells during immune responses are illustrated. The numbers are approximations based on studies of model microbial and other antigens in inbred mice. The relative numbers of viable infectious organisms in an individual over time is indicated by the tan shaded curve (not related to the number of T cells).

Differentiation of Activated T Cells Into Effector Cells

Many of the progeny of the antigen-stimulated T cells differentiate into effector cells. As summarized in the overview of this chapter, effector cells of the CD4$^+$ lineage express surface molecules and secrete cytokines that activate other cells (B lymphocytes, macrophages, and DCs). Whereas naive CD4$^+$ T cells produce mostly IL-2 on activation, effector CD4$^+$ T cells are capable of producing a large number and variety of cytokines that have diverse biologic activities. Effector CD8$^+$ cells are cytotoxic and kill infected cells. Because there are important differences in effector cells of the CD4$^+$ and CD8$^+$ lineages, we will describe their development and functions separately in Chapters 10 and 11.

Development and Properties of Memory T Cells

T cell–mediated immune responses to an antigen usually result in the generation of memory T cells specific for that antigen, which may persist for years, even a lifetime. Memory cells provide effective defense against pathogens that are prevalent in the environment and may be repeatedly encountered. Despite the importance of immunologic memory, many fundamental questions about the generation and maintenance of memory cells have still not been answered.

Memory cells may develop from effector cells along a linear pathway, or effector and memory populations follow divergent differentiation and are two alternative fates of lymphocytes activated by antigen and other stimuli (Fig. 9.13). The mechanisms that determine whether an individual antigen-stimulated T cell will become a short-lived effector cell or enter the long-lived memory cell pool are not fully established. However, as effector T cells contract, a small pool of memory precursor effector cells, often called MPECs, develops from which memory populations are mainly generated. The signals that drive the development of memory cells are also not fully understood. These signals may include the strength of TCR stimulation, the level of costimulation, the cytokine environment, and others. No single transcription factor determines whether an antigen-stimulated T cell will become a terminal effector cell or a memory cell; rather, this choice may be controlled by quantitative differences in numerous transcription factors and epigenetic reprogramming.

Properties of Memory T Cells

The defining properties of memory cells are their ability to survive for prolonged periods after antigen is eliminated and to mount larger and more rapid responses to antigens than do naive cells. Several features of memory cells account for these properties.

- ***Memory cells express increased levels of anti-apoptotic proteins, which may be responsible for their prolonged survival.*** Whereas naive T cells live for weeks or months and are replaced by mature cells that develop in the thymus, memory T cells may survive for years. Thus, as humans age in an environment in which they are constantly exposed and responding to infectious agents, the proportion of memory cells induced by these microbes compared with naive cells progressively increases. In individuals older than 50 years of age, half or more of circulating T cells may be memory cells (see Fig. 2.12). The anti-apoptotic proteins that promote memory cell survival include BCL-2 and BCL-X_L, which block apoptosis induced by a deficiency of survival signals (see Fig. 15.10). The presence of these proteins allows memory cells to survive even after antigen is eliminated and innate immune responses have subsided, when the stimuli for effector T cell survival and proliferation are no longer present.

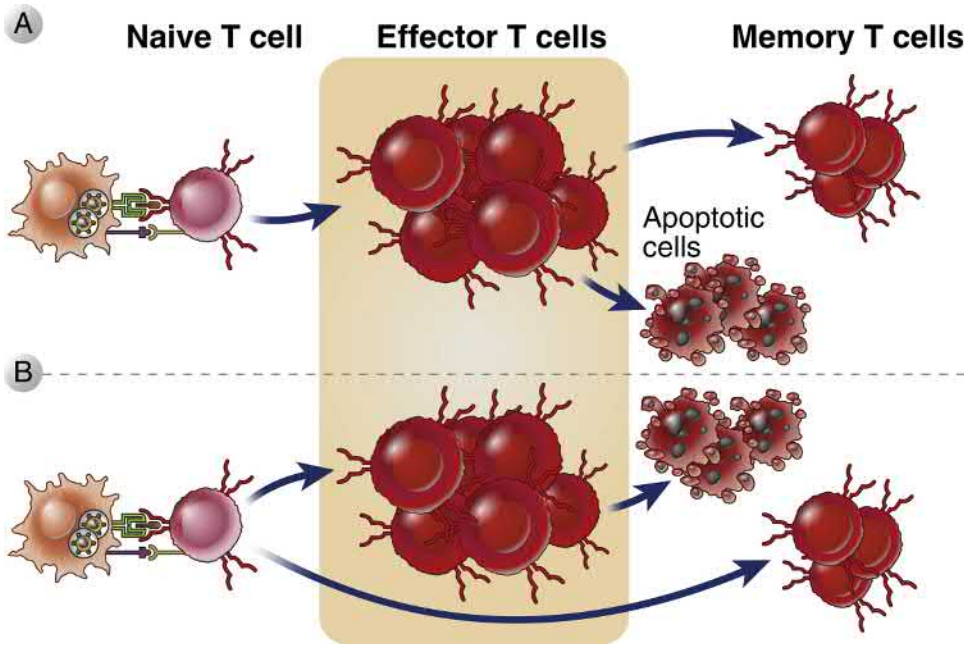


FIGURE 9.13 Development of memory T cells.In response to antigen and costimulation, naive T cells differentiate into effector and memory cells. **A**, According to the linear model of memory T cell differentiation, most effector cells die and some survivors develop into the memory population. **B,** According to the branched differentiation model, effector and memory cells are alternative fates of activated T cells.

- ***Memory cells respond more rapidly to antigen stimulation than do naive cells specific for the same antigen.*** For example, studies in mice have shown that naive T cells differentiate into effector cells in response to antigen in 5 to 7 days, but memory cells acquire effector functions within 1 to 3 days (see Fig. 1.2). This is one reason why secondary responses to antigen exposure are more rapid than primary responses. A possible explanation for this accelerated response is that the gene loci for cytokines and other effector molecules are fixed in an accessible chromatin state in memory cells, in part because of changes in methylation and acetylation of histones. These epigenetically modified genes are poised to respond rapidly to antigen challenge.

- ***The number of memory T cells specific for any antigen is greater than the number of naive cells specific for the same antigen.*** As we discussed earlier, proliferation leads to a large clonal expansion in all adaptive immune responses, and the memory cells that remain from the expanded clone are typically 10- to 100-fold more numerous than the pool of naive cells before antigen encounter. The increased clone size is one reason that antigen challenge in a previously immunized individual induces a larger response than the first immunization in a naive individual.
- ***Memory cells are able to migrate to peripheral tissues and respond to antigens at these sites.*** As we discussed in Chapter 3, naive T cells migrate preferentially to secondary lymphoid organs where they respond to antigens for the first time, but memory cells can migrate to virtually any tissue. These differences are related to differences in the expression of adhesion molecules and chemokine receptors. In addition, memory T cells are less dependent on costimulation than are naive cells, allowing memory cells to respond to antigens presented by a wide range of APCs in peripheral tissues; in contrast, as we have discussed earlier, naive T cells are dependent on antigen presentation by mature DCs in secondary lymphoid organs.
- ***Memory cells undergo slow proliferation, and this ability to self-renew may contribute to the long life span of the memory pool.*** The cycling of these cells may be driven by cytokines. Because of the capacity for self-renewal, memory cells have been likened to stem cells. Although they survive for long periods, memory cells are functionally inactive and have to be restimulated by antigen to become functional effector cells.
- ***The maintenance of memory cells is dependent on cytokines but does not require antigen recognition.*** The most

important cytokine for the maintenance of memory CD4$^+$ and CD8$^+$ T cells is IL-7, which also plays a key role in early lymphocyte development (see Chapter 8) and in the survival of naive T cells (see Chapter 2). Predictably, high expression of the IL-7 receptor (CD127) is characteristic of memory T cells. Memory CD8$^+$ T cells also depend on the related cytokine IL-15 for their survival. IL-7 and IL-15 induce the expression of anti-apoptotic proteins and stimulate low-level proliferation, both of which maintain populations of memory T cells for long periods. The ability of memory cells to survive without antigen recognition has been best demonstrated by experiments in mice in which antigen receptors are genetically deleted after mature lymphocytes have developed. In these mice, the number of naive lymphocytes drops rapidly, but memory cells are maintained.

The most reliable phenotypic markers for memory T cells appear to be the surface expression of the IL-7 receptor and a protein of unknown function called CD27 and the absence of markers of naive and recently activated T cells (see Table 2.5). In humans, most naive T cells express the 200-kD isoform of the surface molecule CD45 called CD45RA (for "restricted A"), and most memory T cells express a 180-kD isoform of CD45 called CD45RO (see Chapter 2).

Both CD4$^+$ and CD8$^+$ memory T cells are heterogeneous and can be subdivided into subsets based on their homing properties and functions. Three major subsets of memory T cells are known.

- **Central memory T cells** (T_{CM}) express the chemokine receptor CCR7 and the adhesion molecule L-selectin and home mainly to lymph nodes. They have a limited capacity to perform effector functions when they encounter antigen, but they undergo brisk proliferative responses and generate many effector cells on antigen challenge. They provide a pool of memory cells that can respond to antigen challenge and develop into effector cells.

- **Effector memory T cells** (T_{EM}), on the other hand, do not express CCR7 or L-selectin, and they home to peripheral sites, especially mucosal tissues. On stimulation by antigen, T_{EM} cells rapidly produce effector cytokines such as IFN-γ or become cytotoxic, but they do not proliferate much. This effector subset, therefore, is poised for a rapid response to exposure to a microbe, but complete eradication of infection may also require large numbers of effectors generated from the pool of central memory T cells. A subset of T_{EM} cells in humans expresses the CD45RA isoform, which is characteristic of naive T cells. This population is called TEMRA cells (T effector memory RA$^+$); whether it has unique functional properties is not known.
- **Tissue-resident memory T cells** (T_{RM}) are present in various nonlymphoid tissues, do not circulate in the blood, and may provide rapid defense against microbes in the tissues. Most of these cells express high levels of CD69, the molecule that reduces expression of S1PR1 (see Chapter 3). As a result, these cells do not respond to the high concentrations of S1P in the lymph and blood, facilitating their retention in tissues. A fourth population of memory T cells, called **peripheral memory T cells** (T_{PM}), has been described. These cells make important contributions to secondary responses in tissues and, unlike T_{RM} cells, are capable of moving between the circulation and tissues.

Memory T cells are also heterogeneous in terms of cytokine profiles. For example, some CD4$^+$ memory T cells may be derived from activated T cells that are not committed to the Th1, Th2, or Th17 phenotype (described in Chapter 10), and when reactivated by exposure to antigen and cytokines, they can differentiate into any of these subsets. Other memory T cells may be derived from differentiated Th1, Th2, or Th17 effectors and retain their respective cytokine profiles on reactivation.

Decline of T Cell Responses

Elimination of antigen leads to contraction of the T cell response, and this decline is responsible for maintaining homeostasis in the immune system. Several mechanisms may account for the decline.

- **Cell death.**As the antigen is eliminated and the innate immune response associated with antigen exposure abates, the signals that normally keep activated lymphocytes alive and proliferating are no longer present. As mentioned earlier, costimulation and growth factors such as IL-2 stimulate expression of the anti-apoptotic proteins BCL-2 and BCL-X_L in the activated lymphocytes, and these proteins keep cells viable. As the level of costimulation and the amount of available IL-2 decrease, the levels of anti-apoptotic proteins in the cells drop. At the same time, growth factor deprivation activates sensors of cellular stress (such as the BH3-only protein BIM), which trigger the mitochondrial pathway of apoptosis and are no longer opposed by the anti-apoptotic proteins (see Fig. 15.10). The net result of these changes is that most of the cells that were produced by activation die and the generation of newly activated cells declines, so the pool of antigen-activated lymphocytes contracts.
- ***Inhibition of T cell activation.*** A number of mechanisms inhibit T cell responses after they have been initiated. These mechanisms likely contribute to physiological downregulation of immune responses to pathogens, and they also are important in maintenance of self tolerance.
  - *Reduced activating signals.* Several intracellular enzymes function to limit the duration or magnitude of T cell activation responses. These include phosphatases such as SHP-1, SHP-2 and SHIP, which dephosphorylate

signaling substrates, and E3 ubiquitin ligases such as CBL-b, which degrade activated signaling intermediates (see Chapter 7). These enzymes may be activated after TCR and costimulatory pathways are engaged.

- ○ *Engagement of cell surface inhibitory molecules.* As mentioned earlier, PD-1 and CTLA-4 are expressed on activated lymphocytes, and both may function to limit continued activation. CTLA-4 upregulation on recently activated T cells may primarily inhibit T cell activation in lymph nodes by competing with CD28 for B7 proteins and thus prevent the generation of effector and memory cells. PD-1 is induced in both activated $CD8^+$ and $CD4^+$ T cells. Its inhibitory function is best understood in the context of restraining previously activated $CD8^+$ T cells in peripheral tissues. It is not clear if these coinhibitors regulate all T cell responses or responses mainly to selected antigens (e.g., for PD-1, some viruses, tumors, and self antigens) (see Chapter 15).
- ○ *Regulatory T cells.* Most immune responses in which effector T cells are activated are accompanied by the development and activation of regulatory T cells. Regulatory T cells function in several ways to inhibit T cell responses, including the engagement of CTLA-4 on their surface with B7 on APCs, as discussed further in Chapter 15. Regulatory T cells limit immunopathology during infection and also suppress autoimmunity and allergy.

Summary

- T cell responses are initiated by signals that are generated by T cell receptor (TCR) recognition of peptide–major histocompatibility complex (MCH) complexes on the surface of antigen-presenting cells (APCs) and through signals provided at the same time by costimulators expressed on APCs.

- The best-defined costimulators are members of the B7 family, which are recognized by receptors of the CD28 family expressed on T cells. The expression of B7 costimulators on APCs is increased by encounter with microbes, providing a mechanism for generating optimal responses against infectious pathogens. Some members of the CD28 family inhibit T cell responses, and the outcome of T cell antigen recognition is determined by the balance between engagement of activating and inhibitory receptors of this family.
- T cell responses to antigen and costimulators include changes in the expression of surface molecules, synthesis of cytokines and cytokine receptors, cellular proliferation, and differentiation into effector and memory cells.
- The surface molecules whose expression is induced on T cell activation include proteins that are involved in retention of T cells in lymphoid organs, cytokines and cytokine receptors, effector and regulatory molecules, and molecules that influence migration of the T cells.
- Shortly after activation, T cells produce the cytokine interleukin-2 (IL-2) and express high levels of the functional IL-2 receptor. IL-2 drives the proliferation of the cells, which can result in marked expansion of antigen-specific clones.
- Some activated T cells may differentiate into memory cells, which survive for long periods and respond rapidly to antigen challenge. The maintenance of memory cells is dependent on cytokines such as IL-7, which may promote the expression of anti-apoptotic proteins and stimulate low-level cycling. Memory T cells are heterogeneous and consist of populations that differ in migration properties and functional responses.
- T cell responses decline after elimination of the antigen, thus returning the system to rest. The decline is because the

signals for continued lymphocyte activation are eliminated and because of various inhibitory mechanisms.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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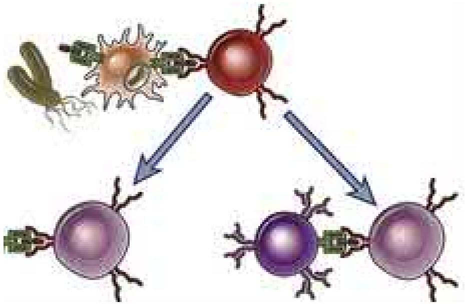
Chapter 10: Differentiation and Functions of CD4$^+$ Effector T Cells



Overview of CD4$^+$ T Cell–Mediated Immune Responses,

Subsets of CD4$^+$ Effector T Cells,
    Properties of Th1, Th2, and Th17 Subsets,
    Development of Th1, Th2, and Th17 Subsets,

The Th1 Subset,
    Development of Th1 Cells,
    Functions of Th1 Cells,

The Th2 Subset,
    Development of Th2 Cells,
    Functions of Th2 Cells,

The Th17 Subset,
    Development of Th17 Cells,
    Functions of Th17 Cells,

Functions of Other HELPER T Cell Subsets,

Other Subsets of CD4+ Effector T Cells,
Other Cytokine-Producing T Cells,
Summary,

Defense against microbes that is mediated by T cells is called **cell-mediated immunity**. T cells provide protection against intracellular and extracellular pathogens and also assist in the elimination of tumor cells. Historically, immunologists divided adaptive immunity into humoral immunity, which (in experiments with animals) can be transferred from an immunized donor to a naive host by antibodies, and cell-mediated immunity, which can be transferred not by antibodies but by T lymphocytes. Humoral immunity neutralizes and eliminates extracellular microbes and toxins that are accessible to antibodies. However, antibodies cannot attack microbes that survive inside phagocytes and other cells. T cell–mediated immunity evolved to provide defense against such microbes. T cells can also enhance killing of microbes that normally live and replicate outside cells but are killed upon ingestion by phagocytes that are activated by T cell–derived cytokines. Therefore, defects in cell-mediated immunity result in increased susceptibility to infection by viruses and bacteria that are obligatory intracellular microbes, as well as some extracellular bacteria and fungi that are eliminated by phagocytes. T cell–mediated reactions are also important in allograft rejection (see Chapter 17), antitumor immunity (see Chapter 18), and hypersensitivity diseases (see Chapter 19).

The two major classes of T cells, $CD4^+$ and $CD8^+$, function in different and complementary ways in cell-mediated immune reactions (Fig. 10.1). The hallmark of $CD4^+$ effector T lymphocytes is that they function mainly through secreted cytokines. They serve a critical role in phagocyte-mediated elimination of microbes, which is the historical definition of cell-mediated immunity. $CD4^+$ T cells also activate other leukocytes, including neutrophils and eosinophils, and stimulate antibody production by B cells. CD8$^+$ effector cells kill infected cells and tumor cells and are responsible for the eradication of microbes, typically viruses, that survive and replicate inside any cell, including nonphagocytic cells. In this chapter, we will describe the role of CD4$^+$ T cells in eliminating microbes. At the end, we will discuss some less numerous populations of T cells whose roles in defense and disease are not as well defined. The differentiation and function of CD8$^+$ effector cells are discussed in Chapter 11.

Overview of CD4$^+$ T Cell–Mediated Immune Responses

The sequence of events in the responses of CD4$^+$ T cells involves the initial activation of these cells in lymphoid organs to generate effector and memory cells, migration of many of these effector cells to sites of infection, and elimination of infectious pathogens at these sites (Fig. 10.2). We described the early steps in the activation of T cells in Chapter 9, and we will describe the generation and functions of effector CD4$^+$ T cells in this chapter.

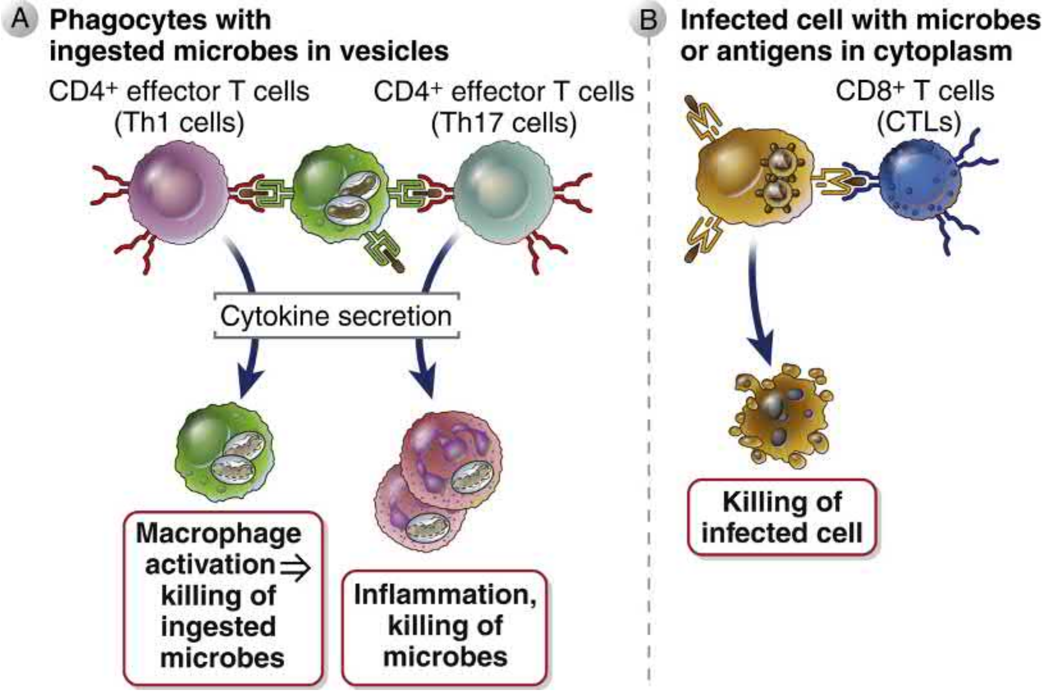

A Phagocytes with ingested microbes in vesicles
CD4+ effector T cells (Th1 cells)
CD4+ effector T cells (Th17 cells)
Cytokine secretion
Macrophage activation⇒ killing of ingested microbes
Inflammation, killing of microbes
B Infected cell with microbes or antigens in cytoplasm
CD8+ T cells (CTLs)
Killing of infected cell

FIGURE 10.1 Role of T cells in eradicating infections. **A,** CD4$^+$ T cells recognize antigens of phagocytosed and extracellular microbes and produce cytokines and cell surface molecules that recruit and activate the phagocytes to kill the microbes. CD8$^+$ T cells can also secrete some cytokines and participate in similar reactions (not shown). **B,** CD8$^+$ cytotoxic T lymphocytes *(CTLs)* recognize antigens of microbes residing in the cytoplasm of infected cells and kill the cells.

Effector CD4$^+$ T cells are generated in secondary lymphoid organs, and most of the effector cells leave these organs and migrate to peripheral sites of infection, where they function to eliminate microbes. This migration of effector T cells to sites of infection is dependent on endothelial adhesion molecules and chemokines expressed at these sites (see Chapter 3). Although migration is largely independent of antigen, T cells that recognize antigen in tissues may be preferentially retained there. Once in the tissues, the T cells encounter microbial antigens presented by macrophages and other antigen-presenting cells (APCs). T cells that specifically recognize antigens receive signals through their antigen receptors that increase the affinity of integrins for their ligands. Two of these integrins, VLA-4 and -5 (very late antigens-4 and -5), bind to fibronectin in extracellular matrices, and a third adhesion molecule, CD44, which is also highly expressed on activated T cells, binds to hyaluronan. In addition, chemokine receptors expressed on activated T cells bind chemokines that are produced in tissues. As a result of these adhesive and chemotactic interactions, antigen-specific effector T cells that encounter the antigen are retained at the extravascular site. T cells not specific for the antigen that migrate into a site of inflammation may die in the tissue or return to the circulation through lymphatic vessels. Some memory T cells also migrate to peripheral tissues, using the same adhesion molecules and chemokine receptors as do effector cells.

A fraction of the $CD4^+$ T cells that are activated in secondary lymphoid organs do not exit the organs but migrate into lymphoid follicles within the organs, where they help B cells to produce high-affinity antibodies of different isotypes. These helper T cells are called T follicular helper (Tfh) cells; their development, properties, and functions in humoral immune responses are described in Chapter 12.

In cell-mediated immune responses against phagocytosed microbes, T cells specifically recognize microbial antigens, but recruited phagocytes and other myeloid cells actually destroy the pathogens. Thus, effector T cells of the $CD4^+$ lineage link specific recognition of microbes with the activation of other leukocytes that destroy the microbes. This fundamental concept was first appreciated from studies of cell-mediated immunity to the intracellular bacterium *Listeria monocytogenes* (Fig. 10.3). It was shown that mice previously infected with a low (sublethal) dose of *Listeria* were protected from challenge with higher doses that were lethal in previously uninfected animals. Protection could be transferred to naive animals with lymphocytes (later shown to be T lymphocytes) from the infected mice but not with serum, the fluid fraction of clotted blood that contains antibodies. These results demonstrated that specific protection against an intracellular bacterial infection was mediated by T cells. However, in vitro, the bacteria were killed not by T cells from immune animals but by activated macrophages, emphasizing the central role of macrophages in microbe elimination. Such studies established that defense against intracellular microbes required cooperative interactions between antigen-specific T cells and microbicidal phagocytes, and we now know this type of interaction is an important component of cell-mediated immunity.

Ingestion and elimination of microbes by phagocytes is also a major reaction of innate immunity, but T cells greatly enhance this function of phagocytes. As we discussed in Chapter 4, phagocytes recognize microbes and are activated by pathogen-associated molecular patterns, such as Toll-like receptor (TLR) ligands, and they are capable of destroying a variety of microbes. However, many infectious pathogens have evolved to resist this mechanism of innate immunity and can survive and even replicate inside macrophages. In such infections, T cells recognize microbial protein antigens and activate phagocytes, enabling them to destroy microbes that may not be eliminated by the innate functions of the macrophages in the absence of T cell help. $CD4^+$ effector T cells activate phagocytes via surface molecules, principally CD40 ligand (CD40L), and secreted cytokines. We will see how these signals cooperate when we discuss the activation of macrophages later in this chapter.

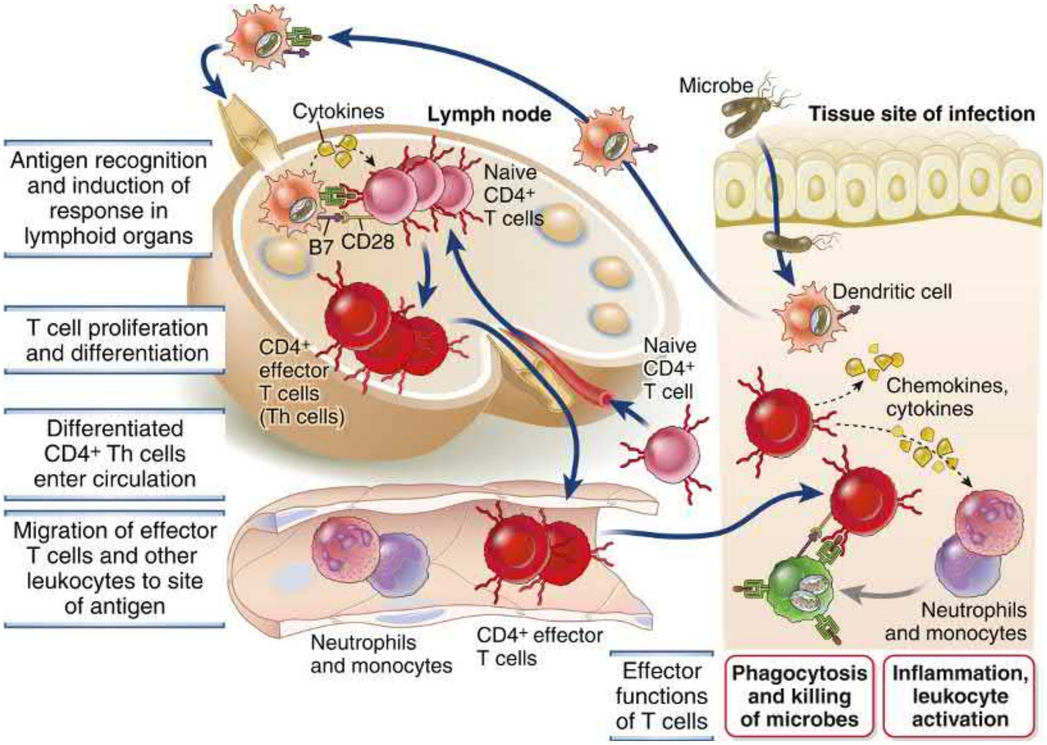

Microbe
Tissue site of infection
Cytokines
Lymph node
Antigen recognition and induction of response in lymphoid organs
Naive CD4+ T cells
B7
CD28
Dendritic cell
T cell proliferation and differentiation
CD4+ effector T cells (Th cells)
Naive CD4+ T cell
Chemokines, cytokines
Differentiated CD4+ Th cells enter circulation
Migration of effector T cells and other leukocytes to site of antigen
Neutrophils and monocytes
Neutrophils and monocytes
CD4+ effector T cells
Effector functions of T cells
Phagocytosis and killing of microbes
Inflammation, leukocyte activation

FIGURE 10.2 Steps in CD4$^+$ T cell–mediated immune responses. CD4$^+$ T cells recognize peptides that are derived from protein antigens and presented by dendritic cells in secondary lymphoid organs. The T lymphocytes are stimulated to proliferate and differentiate into effector (and memory) cells, which enter the circulation and migrate to sites of infection in peripheral tissues. In the tissues, effector T cells recognize the antigen and respond by secreting cytokines that recruit more leukocytes and activate phagocytes to eradicate the infection.

Inflammation, consisting of leukocyte recruitment and activation, accompanies many of the reactions of CD4$^+$ T lymphocytes. This T cell–dependent inflammation serves as an antimicrobial defense mechanism but also can be injurious to tissues. When a T cell reaction causes injury, it is called **delayed-type hypersensitivity (DTH)**, the term hypersensitivity referring to an excessive or damaging immune response. DTH frequently occurs together with protective cell-mediated immunity against microbes and may be the cause of much of the pathology associated with certain types of infectious and autoimmune diseases (see Chapters 16 and 19).

Because the functions of CD4$^+$ T cells are mediated in large part by cytokines, there has been great interest in defining these cytokines, which cells produce them, and how they function. An important discovery in immunology was the identification of populations of CD4$^+$ effector T cells that produce different cytokines and, therefore, perform distinct functions. We will begin with a description of the major properties of these subsets and then describe the development and functions of each population.

Subsets of CD4$^+$ Effector T Cells

There are four major subsets of CD4$^+$ effector T cells, three of which, called Th1, Th2, and Th17, secrete different sets of cytokines, function in host defense against distinct types of infectious pathogens, and are involved in different types of tissue injury in immunologic diseases (Fig. 10.4). The fourth subset, Tfh cells, is important in antibody-mediated immunity and is discussed in Chapter 12. Regulatory T cells are another distinct population of CD4$^+$ T cells. They are not effector cells; rather, their function is to control immune reactions to self and foreign antigens, and they are described in Chapter 15 in the context of immunologic tolerance.

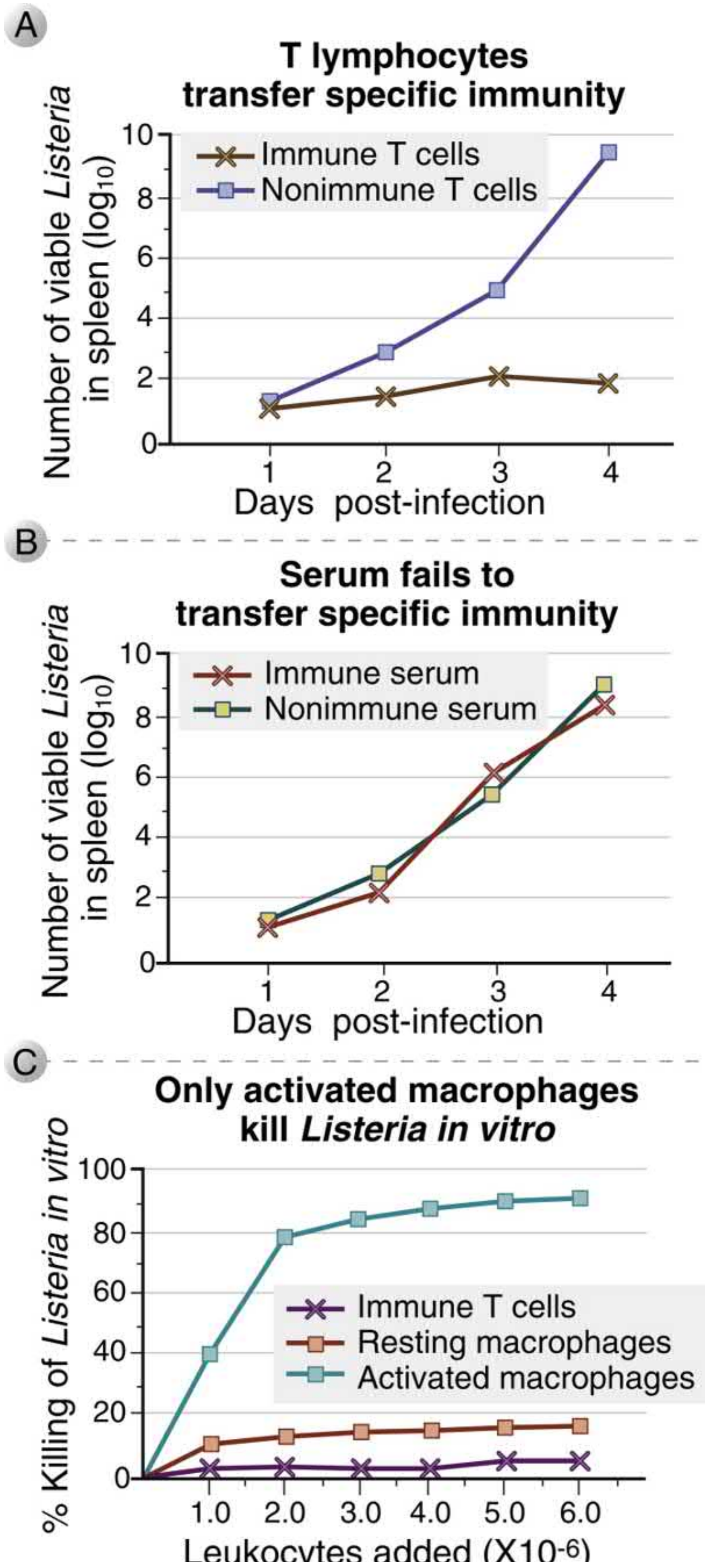

A
T lymphocytes transfer specific immunity
Number of viable *Listeria* in spleen ($\log_{10}$)
10
8
6
4
2
0
Immune T cells
Nonimmune T cells
1
2
3
4
Days post-infection
B
Serum fails to transfer specific immunity
Number of viable *Listeria* in spleen ($\log_{10}$)
10
8
6
4
2
0
Immune serum
Nonimmune serum
1
2
3
4
Days post-infection
C
Only activated macrophages kill *Listeria in vitro*
% Killing of *Listeria in vitro*
100
80
60
40
20
0
Immune T cells
Resting macrophages
Activated macrophages
1.0
2.0
3.0
4.0
5.0
6.0
Leukocytes added ($X10^{-6}$)

FIGURE 10.3 Cell-mediated immunity to *Listeria monocytogenes*.Immunity to *L. monocytogenes* is measured by inhibition of bacterial growth in the spleens of animals inoculated with a known dose of viable bacteria. Such immunity can be transferred to normal mice by T lymphocytes **(A)** but not by serum **(B)** from syngeneic mice previously immunized with killed or low doses of *L. monocytogenes*. In an in vitro assay of cell-mediated immunity, the bacteria are actually killed by activated macrophages and not by T cells **(C)**.

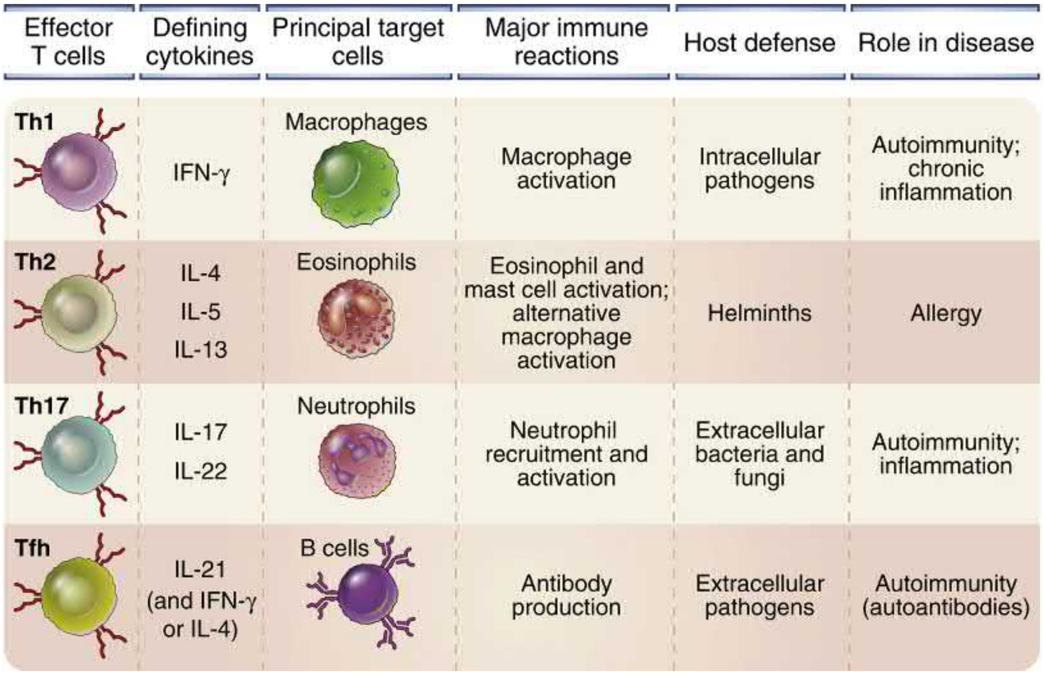
Properties of Th1, Th2, and Th17 Subsets

It was appreciated many years ago that host responses to different infections vary greatly, as do the reactions in different immunologic diseases. For instance, the immune reaction to bacteria that survive within phagocytes, like *Mycobacterium tuberculosis,* is dominated by activated macrophages, whereas the reaction to helminthic parasites consists of the production of immunoglobulin E (IgE) antibody and the activation of eosinophils. Furthermore, in many chronic autoimmune diseases, tissue damage is caused by inflammation with accumulation of neutrophils and macrophages, whereas in allergic disorders, the lesions contain abundant eosinophils along with other leukocytes. The realization that all of these phenotypically diverse immunologic reactions are dependent on $CD4^+$ T cells raised an obvious question: How can the same $CD4^+$ cells elicit such different responses? The answer, as we now know, is that $CD4^+$ T cells consist of subsets of effector cells that produce distinct sets of cytokines, elicit quite different reactions, and are involved in host defense against different microbes, as well as in distinct types of immunologic diseases. The first two subsets that were discovered were called types 1 and 2 helper T cells, or Th1 and Th2. The Th17 subset, so named because its characteristic cytokine is interleukin-17 (IL-17), was identified many years later as the T cells responsible for some CD4$^+$ T cell–mediated inflammatory diseases that could not be attributed to the Th1 and Th2 subsets.

The defining characteristics of differentiated subsets of effector cells are the cytokines they produce, which is related to the transcription factors they express. The transcription factors are responsible for production of different cytokines by these subsets as well as expression of different chemokine receptors and other proteins. These characteristics of each subset are described in the following section.

The signature cytokines produced by the major CD4$^+$ T cell subsets are interferon (IFN)-γ for Th1 cells; IL-4, IL-5, and IL-13 for Th2 cells; and IL-17 and IL-22 for Th17 cells (see Fig. 10.4) **.** The cytokines produced by these T cell subsets determine their effector functions and roles in diseases. Some of the cytokines made by each subset also stimulate the development and expansion of that subset and inhibit differentiation of the other Th subsets, thus contributing to amplification of each type of helper T cell response, a process called polarization (discussed later). The production of distinct sets of cytokines is initiated by the expression of subset-specific transcription factors and is sustained by epigenetic modifications of specific cytokine gene loci. These mechanisms are described later.

Th1, Th2, and Th17 cells have distinct patterns of homing, in large part because they express chemokine receptors and adhesion molecules that direct them to migrate into different sites of infections. We discussed the control of lymphocyte migration in Chapter 3. Th1, but not Th2, cells express high levels of the chemokine receptors CXCR3 and CCR5, which bind to chemokines produced in tissues during innate immune responses. Therefore, Th1 cells tend to be abundant at sites of infection where the infectious agents trigger strong innate immune reactions; these agents include many bacteria and viruses. Th1 cells also express high levels of ligands for E-selectin and P-selectin, which assist in the migration of these cells to sites of strong inflammation (where the selectins are expressed on the endothelium). In contrast, Th2 cells express the chemokine receptors CCR3, CCR4, and CCR8, which recognize chemokines that are highly expressed at sites of helminthic infection or allergic reactions, particularly in mucosal tissues, and so Th2 cells tend to migrate to these tissues. Th17 cells express CCR6, which binds the chemokine CCL20, which is produced by various tissue cells and macrophages in some bacterial and fungal infections.



Effector T cells	Defining cytokines	Principal target cells	Major immune reactions	Host defense	Role in disease
Th1	IFN-γ	Macrophages	Macrophage activation	Intracellular pathogens	Autoimmunity; chronic inflammation
Th2	IL-4 IL-5 IL-13	Eosinophils	Eosinophil and mast cell activation; alternative macrophage activation	Helminths	Allergy
Th17	IL-17 IL-22	Neutrophils	Neutrophil recruitment and activation	Extracellular bacteria and fungi	Autoimmunity; inflammation
Tfh	IL-21 (and IFN-γ or IL-4)	B cells	Antibody production	Extracellular pathogens	Autoimmunity (autoantibodies)

FIGURE 10.4 Properties of the major subsets of $CD4^+$ helper T cells.Naive $CD4^+$ T cells may differentiate into distinct subsets of effector cells in response to antigen, costimulators, and cytokines. Each subset acts mainly on another cell of the immune system (referred to as *target cells*) and serves different functions and roles in disease. T follicular helper *(Tfh)* cells are discussed in Chapter 12. *IL,* Interleukin.

Although for many years it was thought that Th1 and Th2 cells help B lymphocytes to produce different antibodies, it is now clear that, as stated earlier, most of these differentiated effector cells leave the lymphoid organs where they are generated and migrate to peripheral sites of infection. Antibody responses develop mostly in secondary lymphoid organs, and particularly in germinal centers, where antigen-specific B and T cells interact. The CD4$^+$ helper T cells that remain in secondary lymphoid organs to help B lymphocytes are not classical Th1 or Th2 but Tfh cells that make some of the same cytokines that Th1 and Th2 cells do (see Chapter 12).

Different inflammatory diseases are caused by excessive reactions of different helper T cell subsets. In general, Th1 and Th17 cells play prominent roles in autoimmune diseases associated with inflammation, whereas allergic reactions are dominated by Th2 cells.

The identification of Th1, Th2, and Th17 subsets has provided many insights into lymphocyte responses. However, there are some caveats with the idea that all effector CD4$^+$ T cells can be classified into these subsets based on defined criteria. Many CD4$^+$ effector T cells produce combinations of cytokines or only some of the cytokines characteristic of a particular subset and are not readily classifiable into separable populations. For instance, in many inflammatory reactions, there may be individual T cells that produce both IFN-γ (characteristic of Th1 cells) and IL-17 (typical of Th17 cells). Conversely, some cells may produce cytokines that are not characteristic of any of the three subsets (such as IL-9) or only some of the cytokines produced by a particular subset. These restricted cytokine profiles have led to an expanding nomenclature describing these populations (such as Th9, Th22, and so on). It is not known whether cells with mixed or limited cytokine patterns are intermediates in the development of the classical polarized effector cells or are themselves fixed populations.

It is also clear that some of these effector T cells may convert from one cytokine profile to another in response to changes in activation conditions. The extent and significance of plasticity or stability of differentiated effector T cells remain topics of active research.

Although $CD4^+$ effector T cells are the main sources of many cytokines in protective and pathologic adaptive immune responses, the same cytokines may be produced by other cell types, such as γδ T cells and innate lymphoid cells (ILCs). As we discussed in Chapter 4, ILCs are classified into groups that produce many of the same cytokines as subsets of effector $CD4^+$ T cells do. For this reason, the concept has evolved that cytokine-dependent host defense and pathologic reactions are mediated by the coordinated actions of ILCs early in the response and effector $CD4^+$ T cells late. Such responses may be viewed as types of immunity; type 1 immunity relies on ILC1, NK, and Th1 cells; type 2 immunity on ILC2 and Th2 cells; and type 3 immunity on ILC3 and Th17 cells.

Development of Th1, Th2, and Th17 Subsets

Differentiated Th1, Th2, and Th17 cells all develop from naive $CD4^+$ T lymphocytes, mainly in response to cytokines present early during immune responses. The process of effector T cell development involves multiple steps. Signals that T cells receive from APCs and other cells at the site of the immune response initiate the conversion of antigen-stimulated T cells to effector cells. Developing effector cells become progressively committed to a particular cytokine production profile, and cytokines amplify these differentiation pathways. The net result is the progressive accumulation of T cell populations that produce distinct sets of cytokines.

There are several important general features of T cell subset differentiation.

- ***The cytokines that drive the development of $CD4^+$ T cell subsets are produced by APCs (primarily dendritic cells [DCs] and macrophages) and other immune cells (such as NK***

cells and mast cells) present in the lymphoid organ where the immune response is initiated. DCs that encounter microbes and display microbial antigens are activated to produce cytokines (as well as costimulators) as part of innate immune responses to the microbes (see Chapter 4). Different microbes may stimulate DCs to produce distinct sets of cytokines, perhaps because the microbes activate different DC populations or are recognized by different microbial sensors in the cells. Other cells of innate immunity, such as NK cells, ILCs, and mast cells, also produce cytokines that promote the development of antigen-stimulated T cells into different effector subsets.

- ***Stimuli other than cytokines influence the pattern of helper T cell differentiation.*** Experimental evidence indicates that the affinity of the T cell receptor (TCR) for antigen, the amount of antigen, and the nature of the APC all determine the dominant subset that develops after antigen recognition. But the contribution of these factors to the development of effector subsets in most immune responses is not clear. The genetic makeup of the host is another important determinant of the pattern of T cell differentiation. Some inbred strains of mice develop Th2 responses to the same microbes that stimulate Th1 differentiation in most other strains. Strains of mice that develop Th2-dominant responses are susceptible to infections by intracellular microbes (see Chapter 16). It is possible, but not proven, that people differ in their propensity to mount Th1, Th2, or Th17 responses based on inherited genes.
- ***The distinct cytokine profiles of differentiated cell populations are controlled by particular transcription factors that activate cytokine gene expression and by chromatin modifications affecting accessibility of these factors to the promoters and regulatory elements of cytokine genes.*** The transcription factors are activated or induced by signals from the TCR, innate immune receptors, costimulators, and cytokine receptors. Each subset

expresses its own characteristic set of transcription factors. As the subsets become increasingly polarized, the gene loci encoding that subset's signature cytokines undergo histone modifications (such as changes in methylation and acetylation) and other chromatin remodeling events, so that these loci remain accessible to RNA polymerase and transcription factors, whereas the loci for other cytokines (those not produced by that subset) are in an inaccessible chromatin state. Thus, the cytokine genes characteristic of a particular subset become fixed in an antigen responsive state, whereas genes that encode cytokines not produced by that subset remain inactive. These epigenetic changes are inherited in the progeny of proliferating cells, thus ensuring that the effector T cells become stably committed to producing a particular set of cytokines.

- ***Each subset of differentiated effector cells produces cytokines that promote its own development and may suppress the development of the other subsets.*** This feature of T-cell subset development provides a powerful amplification mechanism. For instance, IFN-γ secreted by Th1 cells stimulates further Th1 differentiation and inhibits the generation of Th2 and Th17 cells. Similarly, IL-4 produced by Th2 cells promotes Th2 differentiation. Thus, once an immune response develops along one effector pathway, it becomes increasingly polarized in that direction, and the most extreme polarization is seen in chronic infections or upon chronic exposure to environmental antigens, when the immune stimulation is prolonged.
- ***Differentiation of each subset is induced by the types of microbes that the subset is best able to combat.*** For instance, the development of Th1 cells is driven by intracellular microbes, against which the principal defense is Th1 mediated. By contrast, the immune system responds to helminthic parasites by the development of Th2 cells, and the cytokines produced by these cells are important for combating helminths. Similarly, Th17 responses are

induced by some bacteria and fungi and are most effective at defending against these pathogens. The generation and effector functions of these differentiated T cells are an excellent illustration of the concept of specialization of adaptive immunity, which refers to the ability of the immune system to respond to different microbes in ways that are optimal for combating those microbes.

With this background, we will proceed to a description of the development and functions of each subset.

The Th1 Subset

The IFN-γ–producing Th1 subset is induced by microbes that are ingested by and have evolved to survive and replicate within phagocyte; it is the major effector T cell population in phagocyte-mediated host defense. Th1 cells were the first defined subset of helper T cells shown to mediate cellular immunity against pathogens that survive inside phagocytes.

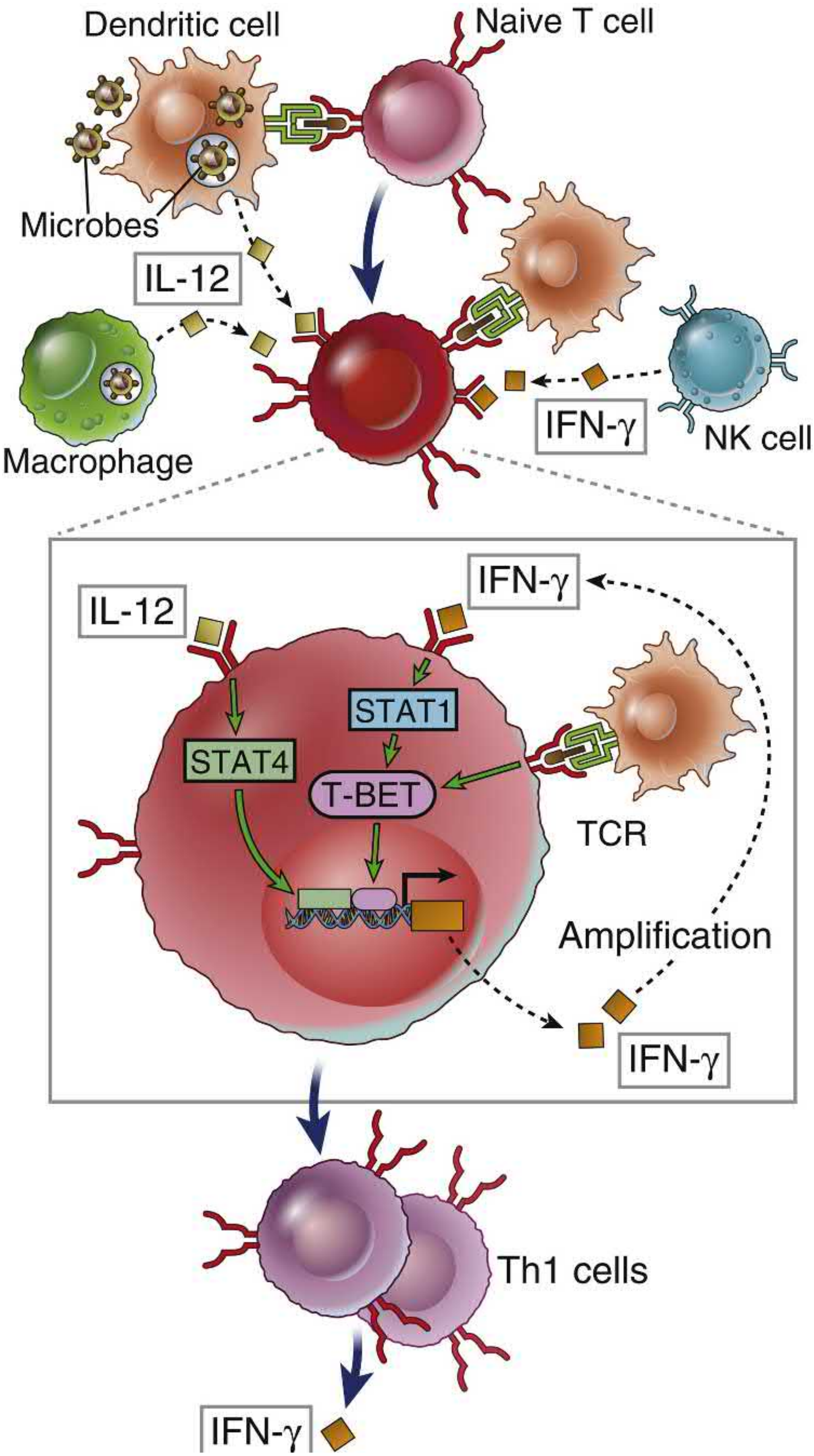

Dendritic cell
Naive T cell
Microbes
IL-12
Macrophage
IFN-γ
NK cell
IL-12
IFN-γ
STAT1
STAT4
T-BET
TCR
Amplification
IFN-γ
Th1 cells
IFN-γ

FIGURE 10.5 Development of Th1 cells.Interleukin-12 *(IL-12)* produced by dendritic cells and macrophages in response to microbes, including intracellular microbes, and interferon-γ *(IFN-γ)* produced by natural killer *(NK)* cells (all part of the early innate immune response to the microbes) activate the transcription factors T-BET, STAT1, and STAT4, which stimulate the differentiation of naive CD4$^+$ T cells to the Th1 subset. IFN-γ produced by the Th1 cells amplifies this response and inhibits the development of Th2 and Th17 cells. Other cytokines, including type I IFNs and IL-18, also promote Th1 differentiation but are not shown.

Development of Th1 Cells

Th1 differentiation is driven mainly by the cytokines IL-12 and IFN-γ and occurs in response to microbes that activate DCs, macrophages and NK cells (Fig. 10.5) . The differentiation of antigen-activated CD4$^+$ T cells to Th1 effectors is stimulated by many intracellular bacteria, such as *Listeria* and mycobacteria, and by some parasites, such as *Leishmania,* all of which infect DCs and macrophages. Th1 differentiation is also stimulated by viruses and by protein antigens administered with strong adjuvants. A common feature of these infections and immunization conditions is that they elicit innate immune reactions that are associated with the production of certain cytokines, including IL-12, IL-18, and type I IFNs. All of these cytokines promote Th1 development; of these, IL-12 is probably the most potent. Many microbes stimulate NK cells to produce IFN-γ, which is itself a strong Th1-inducing cytokine and also acts on DCs and macrophages to induce more IL-12 secretion. After Th1 cells have developed, they secrete IFN-γ, which promotes more Th1 differentiation and thus amplifies the reaction.

In addition, IFN-γ inhibits the differentiation of naive CD4$^+$ T cells to the Th2 and Th17 subsets, thus promoting the polarization of the immune response in one direction. T cells may further enhance cytokine production by DCs and macrophages by virtue of CD40L on activated T cells engaging CD40 on the APCs and stimulating IL-12 secretion.

IFN- γ and IL-12 stimulate Th1 differentiation by inducing and activating the transcription factors T-BET, STAT1, and STAT4 (see Fig. 10.5) **.** T-BET, a member of the T-box family of transcription factors, is induced in naive CD4$^+$ T cells in response to antigen and IFN-γ. IFN-γ also activates STAT1 (signal transducer and activator of transcription 1), which in turn stimulates expression of T-BET. T-BET then promotes IFN-γ production through a combination of direct transcriptional activation of the *IFNG* gene and by inducing chromatin remodeling of the *IFNG* promoter region. The ability of IFN-γ to stimulate T-BET expression and the ability of T-BET to enhance IFN-γ transcription set up a positive amplification loop that drives differentiation of T cells toward the Th1 phenotype. IL-12 contributes to Th1 commitment by binding to receptors on antigen-stimulated CD4$^+$ T cells and activating the transcription factor STAT4, which further enhances IFN-γ production.

Functions of Th1 Cells

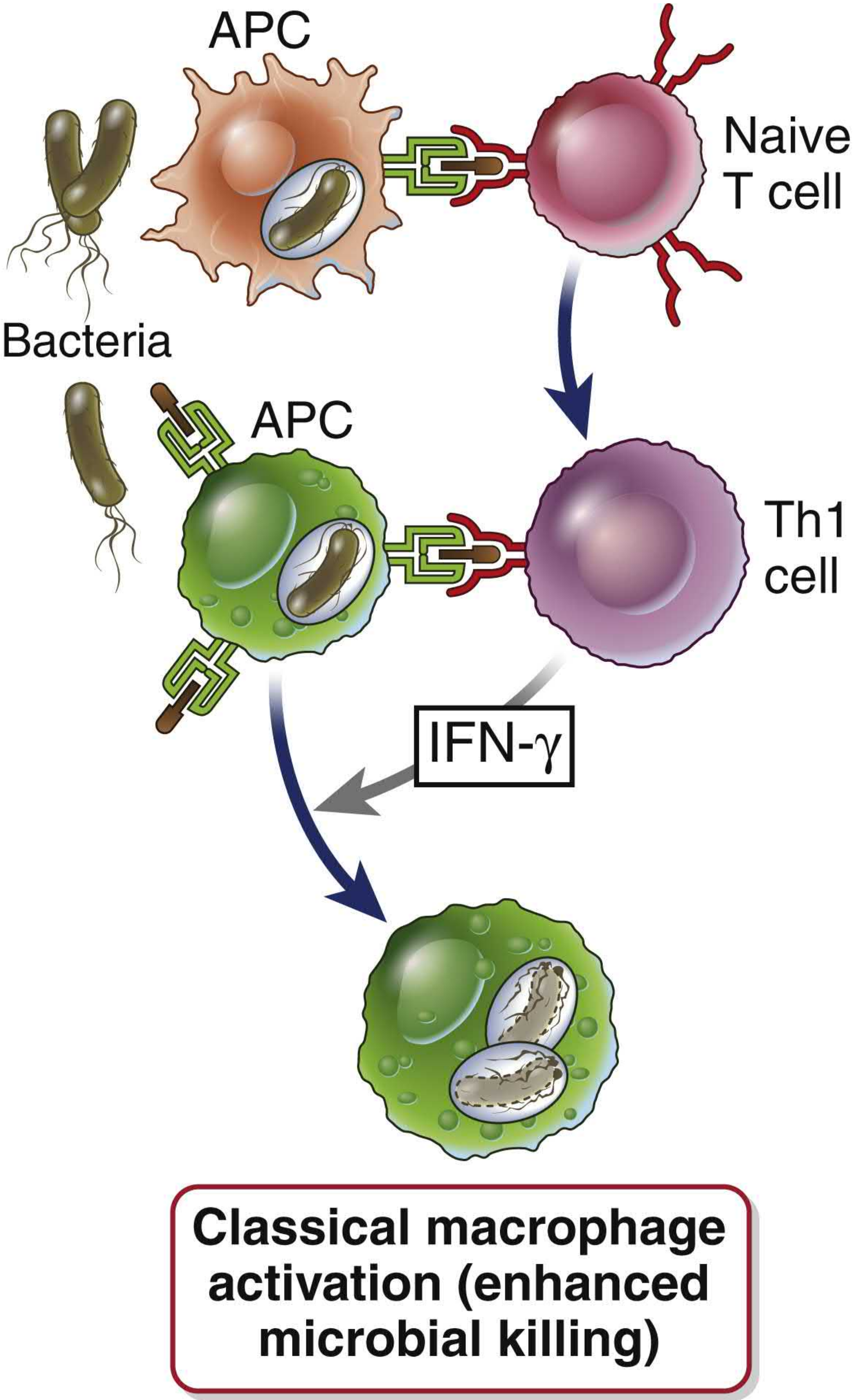
The principal function of Th1 cells is to activate macrophages to ingest and destroy microbes (Fig. 10.6) **.** The same reaction of Th1-mediated macrophage activation is involved in injurious DTH, which is a component of many inflammatory diseases. Some of these conditions are characterized by granulomatous inflammation, including infectious diseases (such as tuberculosis) and chronic inflammatory diseases (such as sarcoidosis) (see Chapter 19).

Before discussing the activation of macrophages and how they destroy microbes, we will describe the properties of IFN-γ, the cytokine responsible for most of the specialized functions of Th1 cells.

Interferon-γ

IFN-γ is the principal macrophage-activating cytokine. IFN-γ is also called immune or type II IFN. Although its name interferon is shared with the antiviral type I IFNs, it is not a potent antiviral cytokine, and it functions mainly as an activator of effector cells of the immune system.

IFN-γ is a homodimeric protein belonging to the type II cytokine family (see Chapter 7). In addition to $CD4^+$ Th1 cells, ILC1s, NK cells, and $CD8^+$ T cells also produce IFN-γ. NK cells secrete IFN-γ in response to activating ligands on the surface of infected or stressed host cells (see Chapter 4) or in response to IL-12; in this setting, IFN-γ functions as a mediator of innate immunity. In adaptive immunity, T cells produce IFN-γ in response to antigen recognition, and production is enhanced by IL-12 and IL-18.


APC
Naive T cell
Bacteria
APC
Th1 cell
IFN-γ

Classical macrophage activation (enhanced microbial killing)

FIGURE 10.6 Functions of Th1 cells. Th1 cells secrete interferon-γ *(IFN-γ),* which acts on macrophages to increase phagocytosis and killing of microbes in phagolysosomes. Th1 cells also produce tumor necrosis factor, which activates neutrophils and promotes inflammation (not shown). *APC,* Antigen-presenting cell.

The receptor for IFN-γ is composed of two structurally homologous polypeptides belonging to the type II cytokine receptor family, called IFNγR1 and IFNγR2. IFN-γ binds to and induces the dimerization of the two receptor chains. This leads to activation of the associated Janus kinases JAK1 and JAK2 and ultimately to phosphorylation and dimerization of STAT1, which stimulates transcription of several genes (see Chapter 7). IFN-γ–induced genes encode many different molecules that mediate the biologic activities of this cytokine, described next.

- ***IFN-γ activates macrophages to kill phagocytosed microbes.*** IFN-γ works with other signals to induce the type of macrophage activation that is called classical activation, discussed in more detail later.
- ***IFN-γ promotes the differentiation of CD4$^+$ T cells to the Th1 subset and inhibits the development of Th2 and Th17 cells.*** These effects of IFN-γ are because it induces the expression of transcription factors needed for Th1 development and inhibits transcription factors involved in Th2 and Th17 differentiation.
- ***IFN-γ stimulates expression of several different proteins that contribute to enhanced antigen presentation and T cell activation*** (see Fig. 6.8) **.** These proteins include major histocompatibility complex (MHC) molecules; many proteins involved in antigen processing, including components of the proteasome; and B7 costimulators on APCs.

- ***IFN-γ activates macrophages, DCs, and other cells to produce cytokines that amplify the host response.*** These cytokines include TNF, IL-1 and chemokines, which recruit more leukocytes and induce inflammation, and IL-12, which provides a positive feedback loop for IFN-γ production and Th1 development.

Other Th1 Cytokines

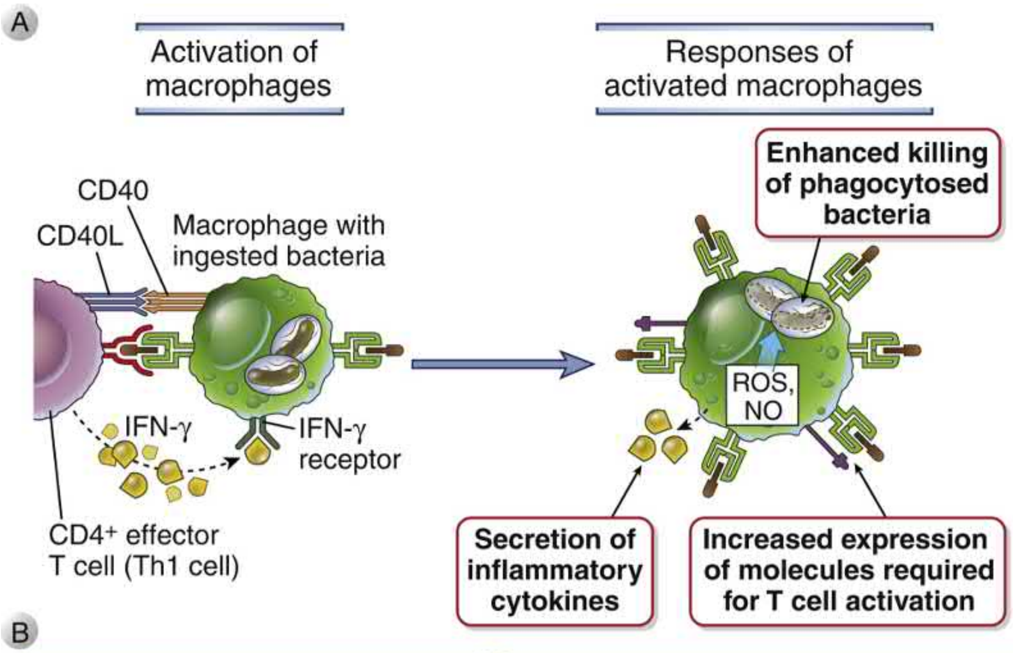
In addition to IFN-γ, Th1 cells produce tumor necrosis factor (TNF), which contributes to the recruitment of leukocytes and enhanced inflammation. Somewhat surprisingly, Th1 cells are also sources of IL-10, which functions mainly to inhibit DCs and macrophages and thus to suppress Th1 activation. This is an example of a negative feedback loop in T cell responses.

Th1-Mediated Classical Macrophage Activation and Killing of Phagocytosed Microbes

Th1 cells activate macrophages through contact-mediated signals delivered by CD40L-CD40 interactions and by IFN-γ (Fig. 10.7) **.** This pathway of macrophage activation is called **classical macrophage activation,** to distinguish it from alternative macrophage activation induced by Th2 cytokines, described later. Classically activated macrophages are also called M1 macrophages. When Th1 cells are stimulated by antigen, the cells express CD40L on their surface and secrete IFN-γ. The actions of IFN-γ on macrophages, described earlier, synergize with the actions of CD40L, and together they are potent stimuli for macrophage activation. CD40 signals activate the transcription factors NF-κB (nuclear factor κB) and AP1 (activation protein 1), and, as discussed earlier, IFN-γ activates the transcription factor STAT1. These transcription factors together induce the expression of genes encoding several enzymes that localize in phagolysosomes of macrophages, including inducible nitric oxide synthase (iNOS), which stimulates the production of nitric oxide (NO), and lysosomal enzymes. Macrophage activation is also associated with the assembly of the enzyme phagocyte oxidase in the membrane of the phagolysosome, which induces the production of reactive oxygen species (ROS) (although this is less prominent than in neutrophils). The requirement for interactions between the surface molecules CD40 on the macrophages and CD40L on the T cells ensures that macrophages that are presenting antigens to the T cells (i.e., the macrophages that are harboring intracellular microbes) are also the macrophages that will be in contact with T cells and thus most efficiently activated by the T cells.

Activated macrophages kill phagocytosed microbes mainly by the actions of NO, ROS, and lysosomal enzymes. All of these potent microbicidal agents are produced within the lysosomes of macrophages and kill ingested microbes after phagosomes fuse with lysosomes (see Fig. 4.17). These toxic substances also may be released into adjacent tissues, where they kill extracellular microbes and may damage normal tissues.

Inherited immunodeficiencies have established the critical importance of Th1 cells in cell-mediated immunity against intracellular pathogens. Different homozygous mutations affecting the IFN-γ receptor, IL-12, IL-12 receptor, and STAT1 cause defects in the development of Th1 cells. Patients with these inherited mutations are susceptible to infection with *Mycobacterium tuberculosis,* low-virulence environmental mycobacteria, and the attenuated *Mycobacterium bovis* strain used in the bacillus Calmette-Guérin (BCG) vaccine. As a group, these disorders are called **Mendelian susceptibility to mycobacterial disease.** The patients are also susceptible to infections with other intracellular bacteria, such as *Salmonella,* and protozoan parasites, further establishing the critical role of the Th1 response in defense against intracellular microbes. Rare patients make autoantibodies against their own IFN-γ and are also susceptible to mycobacterial infections. Humans with inherited mutations in CD40L **(X-linked hyper-IgM syndrome)** and mice in which the gene for CD40 or CD40L is knocked out are highly susceptible to infections with intracellular microbes, notably the fungus *Pneumocystis jiroveci* (see Chapter 21), which require T cell–dependent macrophage activation to be eradicated. These patients and knockout mice also have defects in helper T cell–dependent antibody production, because of the critical role of the CD40L-CD40 interaction in B cell activation (see Chapter 12).


A
Activation of macrophages
Responses of activated macrophages
CD40
CD40L
Macrophage with ingested bacteria
IFN-γ
IFN-γ receptor
CD4+ effector T cell (Th1 cell)
Enhanced killing of phagocytosed bacteria
ROS, NO
Secretion of inflammatory cytokines
Increased expression of molecules required for T cell activation

B

Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment (inflammation) IL-12: Th1 differentiation, IFN-γ production
Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification of T cell response)

FIGURE 10.7 Macrophage activation by Th1 cells. **A,** Macrophages are activated by CD40L-CD40 interactions and by interferon-γ *(IFN-γ)* expressed by Th1 cells and perform several functions that kill microbes, stimulate inflammation, and enhance the antigen-presenting capacity of the cells. **B,** The principal responses of macrophages activated by the classical activation pathway, and their roles in T cell–mediated host defense, are listed. Macrophages are also activated during innate immune reactions and perform similar functions (see Chapter 4). *IL,* Interferon; *MHC,* major histocompatibility; *NO,* nitric oxide; *ROS,*

reactive oxygen species; *TNF*, tumor necrosis factor.

Macrophages activated by Th1 cells are involved in several other reactions of host defense (see Fig. 10.7). They stimulate inflammation mainly through the secretion of cytokines, including TNF and IL-1, which recruit more leukocytes, enhancing the host's ability to destroy infectious pathogens. Activated macrophages may amplify cell-mediated immune responses by becoming more efficient APCs because of increased levels of molecules involved in antigen processing and increased surface expression of class II MHC molecules and costimulators, and by producing cytokines (such as IL-12) that stimulate T lymphocyte differentiation into effector cells.

Some tissue injury may normally accompany Th1 cell–mediated immune reactions to microbes because the microbicidal products released by activated macrophages and neutrophils are capable of injuring normal tissue and do not discriminate between microbes and host tissue. This tissue injury usually resolves as the infection is cleared. However, excessive Th1 reactions are the cause of tissue injury in many chronic inflammatory diseases (see Chapter 19).

The Th2 Subset

Th2 cells activate defense mechanisms that use IgE antibodies, eosinophils, and mast cells to combat microbes. These reactions are important for the eradication of helminthic infections and perhaps also for elimination of other microbes in mucosal tissues. They are central to the development of allergic diseases (see Chapter 20). Th2 cells are also thought to be important in tissue repair.

Development of Th2 Cells

Th2 differentiation occurs in response to helminths and allergens and is dependent on the cytokine IL-4 (Fig. 10.8) . Because the major cytokine that promotes Th2 development is IL-4 and this is a product of Th2 cells, there has been some uncertainty about the source of IL-4 to initiate Th2 responses. The cytokine may be made by antigen-stimulated T cells, by mast cells, and possibly by group 2 innate lymphoid cells (ILC2; see Chapter 4), and other cells in the vicinity of the activated T cells. Other cytokines that may promote the development of Th2 cells include IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), all of which are produced by damaged epithelial and other cells and are involved in the activation of ILC2.

IL-4 stimulates Th2 development by activating the transcription factor STAT6, which, together with TCR signals, induces expression of GATA3 (see Fig. 10.8) **.** GATA3 is a transcription factor that is required for expression of the Th2 cytokines IL-4, IL-5, and IL-13. GATA3 works by directly interacting with the promoters of these cytokine genes and also by causing chromatin remodeling, which opens up the locus for accessibility to other transcription factors. This is similar to the way in which T-BET influences IFN-γ expression. GATA3 functions to stably commit differentiating cells toward the Th2 phenotype, enhancing its own expression through a positive feedback loop. Furthermore, GATA3 blocks Th1 differentiation by inhibiting expression of the signaling chain of the IL-12 receptor. Knockout mice lacking IL-4, STAT6, or GATA3 are deficient in Th2 responses.

Functions of Th2 Cells

Th2 cells stimulate IgE-, mast cell–. and eosinophil-mediated reactions that serve to eradicate helminthic infections and to promote tissue repair (Fig. 10.9) **.** Helminths are too large to be phagocytosed by neutrophils and macrophages and may be more resistant to the microbicidal activities of these phagocytes than are most bacteria and viruses. Therefore, other mechanisms are needed for defense against helminthic infections. The functions of Th2 cells are mediated by IL-5, which activates eosinophils, and IL-13, which has diverse actions. Tfh cells that produce IL-4 and IL-13 stimulate the production of IgE antibodies, which are involved in most Th2-

mediated defense reactions. We will first describe the properties of these cytokines and then their roles in host defense.

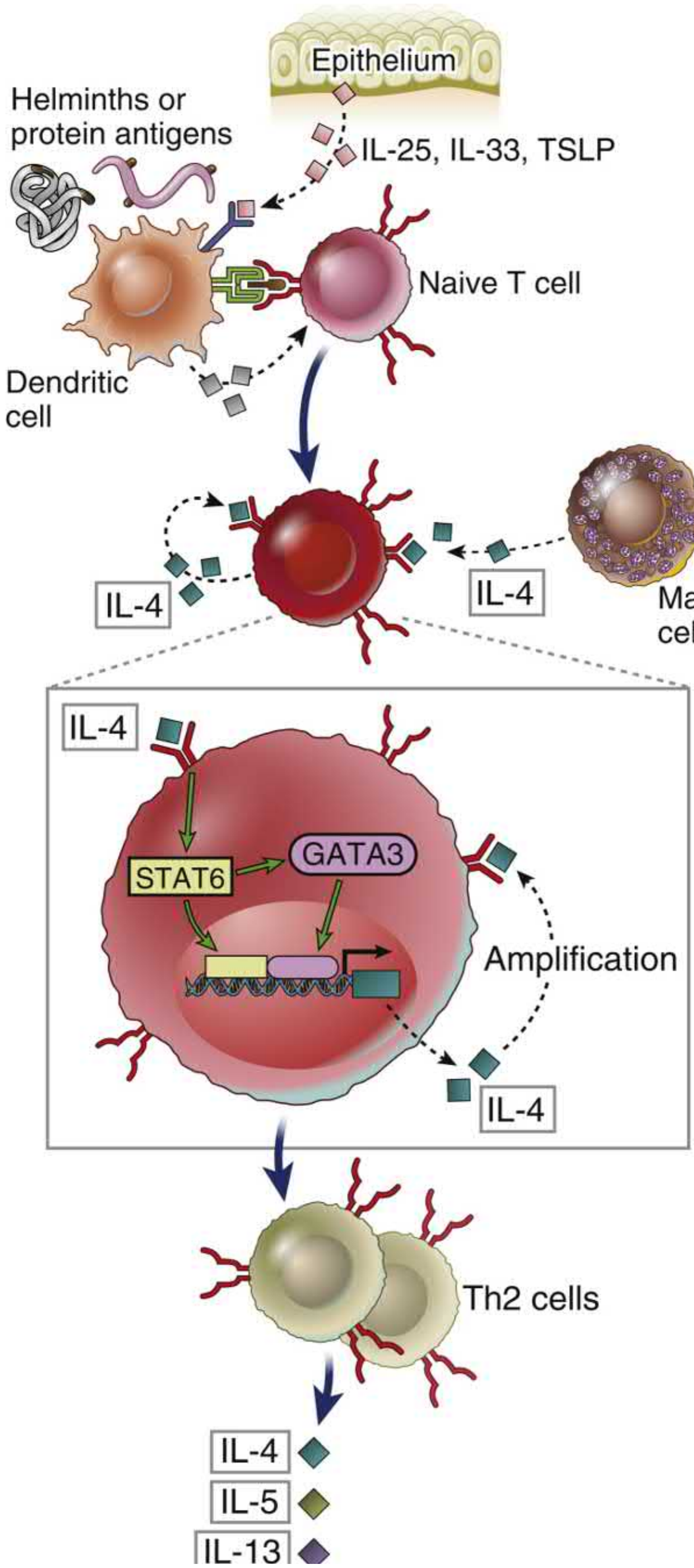

Epithelium
Helminths or protein antigens
IL-25, IL-33, TSLP
Naive T cell
Dendritic cell
IL-4
IL-4
Mast cells
IL-4
STAT6
GATA3
Amplification
IL-4
Th2 cells
IL-4
IL-5
IL-13

FIGURE 10.8 Development of Th2 cells. Dendritic cells may respond to cytokines produced in epithelia by becoming Th2 inducers, by mechanisms that are not well defined. Interleukin-4 *(IL-4)* produced by activated T cells themselves or by mast cells and eosinophils, especially in response to helminths, activates the transcription factors GATA3 and STAT6, which stimulate the differentiation of naive $CD4^+$ T cells to the Th2 subset. IL-4 produced by the Th2 cells amplifies this response and inhibits the development of Th1 and Th17 cells. *TSLP,* Thymic stromal lymphopoietin.

Interleukin-4

IL-4 is the signature cytokine of the Th2 subset and functions as both an inducer and an effector cytokine of these cells. It is a member of the type I four–α-helical cytokine family. The principal cellular sources of IL-4 are $CD4^+$ T lymphocytes of the Th2 subset, ILC2s, and activated mast cells. The IL-4 receptor consists of a cytokine-binding α chain that is a member of the type I cytokine receptor family, associated with the γc chain shared by other cytokine receptors. This IL-4R$\alpha\gamma c$ receptor signals by a JAK-STAT pathway involving JAK1, JAK3, and STAT6, and by a pathway that involves the insulin response substrate (IRS) protein called IRS2. Activated STAT6 induces transcription of genes that account for many of the actions of this cytokine. IL-4 and IL-13 receptors share one of their two chains (described later).

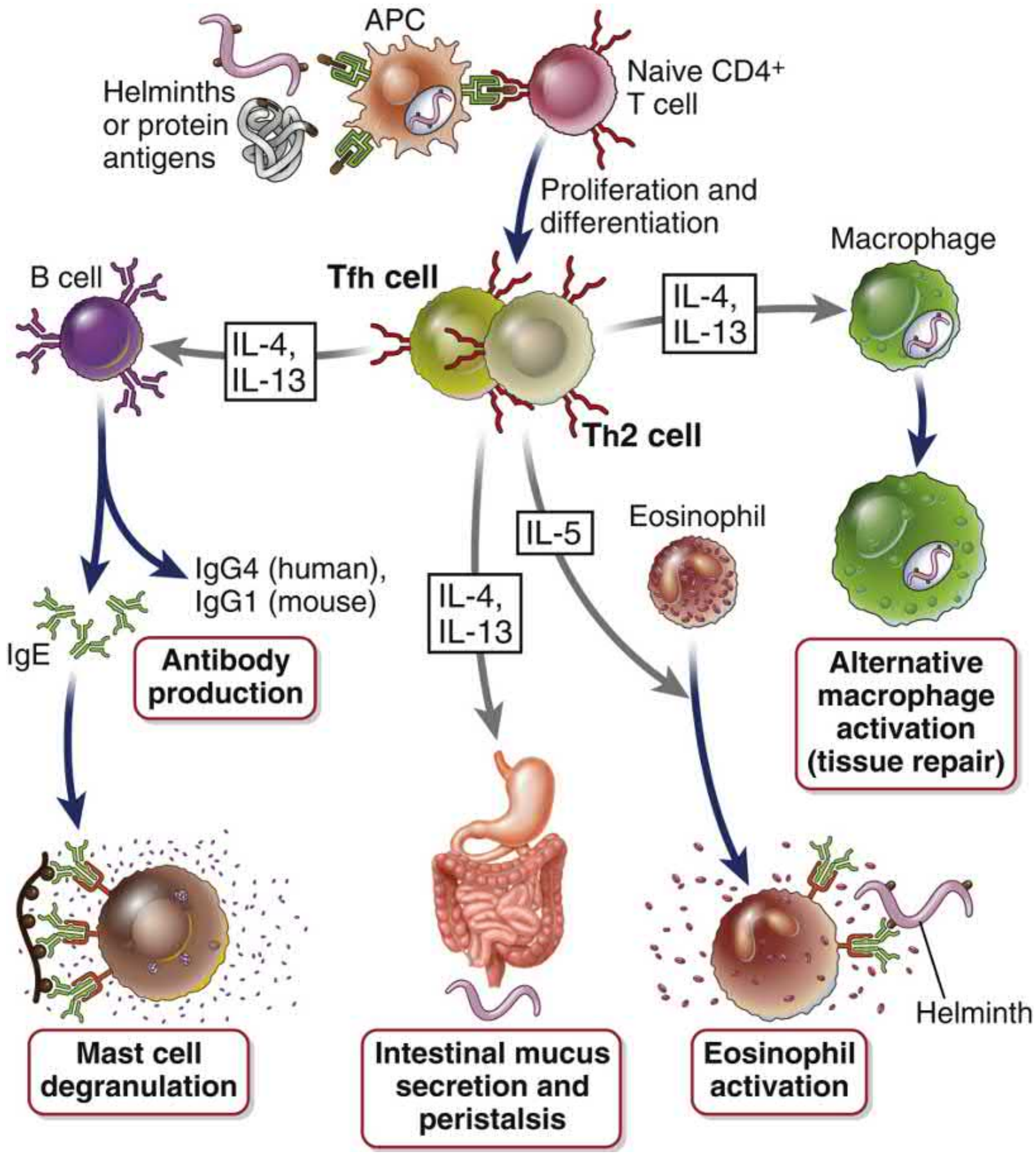

APC
Helminths or protein antigens
Naive CD4+ T cell
Proliferation and differentiation
Macrophage
B cell
Tfh cell
IL-4, IL-13
IL-4, IL-13
Th2 cell
IL-5
Eosinophil
IgG4 (human), IgG1 (mouse)
IL-4, IL-13
IgE
Antibody production
Alternative macrophage activation (tissue repair)
Mast cell degranulation
Intestinal mucus secretion and peristalsis
Eosinophil activation
Helminth

FIGURE 10.9 Functions of Th2 cells.CD4$^+$ T cells that differentiate into Th2 cells secrete IL-4, IL-5, and IL-13. IL-4 (and IL-13) act on B cells to stimulate production of antibodies that bind to mast cells and eosinophils, such as IgE. Help for antibody production may be provided by T follicular helper *(Tfh)* cells that produce Th2 cytokines and reside in lymphoid organs, and not by classical Th2 cells. IL-5 activates eosinophils, a response that is important for defense against helminthic infections. IL-4 and IL-13 are involved in

immunity at mucosal barriers, induce an alternative pathway of macrophage activation, and inhibit classical Th1-mediated macrophage activation. *APC,* Antigen-presenting cell; *Ig,* immunoglobulin; *IL,* interleukin

IL-4 has important actions on several cell types.

- ***IL-4 (and IL-13) produced by Tfh cells stimulates B cell Ig heavy chain class switching to the IgE isotype.*** The mechanisms of class switching are described in Chapter 12. IgE is the principal mediator of immediate hypersensitivity (allergic) reactions (see Chapter 20). IL-4 also enhances switching to IgG4 (in humans, or the homologous IgG1 in mice), but the significance of this role of IL-4 is unclear because these IgG subclasses do not bind to phagocyte Fc receptors or activate complement (see Chapter 13).
- ***IL-4 stimulates the development of Th2 effector cells from naive CD4$^+$ T cells and may be a growth factor for differentiated Th2 cells.*** This function of IL-4 was described earlier.
- ***IL-4, together with IL-13, contributes to an alternative form of macrophage activation that is distinct from the macrophage response to IFN-γ.*** IL-4 and IL-13 suppress IFN-γ–mediated classical macrophage activation and thus inhibit defense against intracellular microbes that are destroyed by phagocytosis. The alternative macrophage activation pathway is described later.
- ***IL-4 (and IL-13) stimulates peristalsis in the gastrointestinal tract, and IL-13 increases mucus secretion from airway and gut epithelial cells.*** Both actions contribute to elimination of microbes at epithelial surfaces.
- ***IL-4 and IL-13 stimulate the recruitment of leukocytes,*** notably eosinophils, by promoting the expression of adhesion molecules on endothelium and the secretion of

chemokines that bind chemokine receptors expressed on eosinophils.

Interleukin-13

IL-13 is structurally and functionally similar to IL-4 and also plays a role in defense against helminths (see Chapter 16) and in allergic diseases (see Chapter 20). IL-13 is a member of the type I four–α-helical cytokine family. IL-13 is produced mainly by the Th2 subset, but group 2 ILCs and other leukocytes may also produce the cytokine. The IL-13 receptor is a heterodimer of the IL-4Rα chain and the IL-13Rα1 chain. This complex can bind both IL-4 and IL-13 with high affinity and also signals through a JAK1, JAK3, and STAT6 pathway. The receptor is expressed on a wide variety of cells, including B cells, mononuclear phagocytes, DCs, eosinophils, basophils, fibroblasts, endothelial cells, and bronchial epithelial cells.

IL-13 works together with IL-4 in defense against helminths and in allergic inflammation. Some of the actions of IL-13 overlap those of IL-4, and others are distinct. Both IL-13 and IL-4 can activate B cells to switch to IgE and some IgG isotypes and recruit leukocytes, and both are involved in alternative macrophage activation. IL-13 stimulates mucus production by airway epithelial cells, an important component of allergic reactions, such as asthma. Unlike IL-4, IL-13 is not involved in Th2 differentiation.

Interleukin-5

IL-5 is an activator of eosinophils and serves as the principal link between T cell activation and eosinophilic inflammation. It is a homodimer of a polypeptide containing a four–α-helical domain and is a member of the type I cytokine family. It is produced mainly by Th2 cells and ILC2s. The IL-5 receptor is a heterodimer composed of a unique α chain and a common β chain (βc), which is also part of the IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors (see Fig. 7.23). The major IL-5–induced signaling pathway involves JAK2 and STAT3.

The principal actions of IL-5 are to activate mature eosinophils and to stimulate the growth and differentiation of eosinophils. Activated eosinophils are able to kill helminths. Eosinophils express Fc receptors specific for IgA and some IgG antibodies and are thereby able to bind to microbes, such as helminths, that are coated with these antibodies.

Roles of Th2 Cells in Host Defense

Th2 cells function in defense against helminthic and other infections by several mechanisms (see Fig. 10.9).

- ***IgE- and eosinophil-mediated reactions.*** IL-4 and IL-13 stimulate the production of helminth-specific IgE antibodies, which may activate mast cells at the site of infection. IL-5 activates eosinophils, and these cells release their granule contents, including major basic protein and major cationic protein, which are capable of destroying even the tough integuments of helminths (see Chapter 16). In allergic reactions, IgE coats mast cells and induces their degranulation upon encounter with antigen (see Chapter 20).
- ***Host defense at mucosal barriers.*** Cytokines produced by Th2 cells are involved in blocking entry and promoting expulsion of microbes from mucosal organs by stimulating mucus production and intestinal peristalsis. Thus, Th2 cells play an important role in host defense at the barriers with the external environment, sometimes called barrier immunity.
- ***Alternative macrophage activation and tissue repair.*** IL-4 and IL-13 activate macrophages to express enzymes that promote collagen synthesis and fibrosis. The macrophage response to Th2 cytokines has been called **alternative macrophage activation** (Fig. 10.10) to distinguish it from the activation induced by IFN-γ, which was characterized first (and hence the designation classical) and which results in potent microbicidal functions and inflammation (see Fig.

10.7). Alternatively activated (also called M2) macrophages produce cytokines that terminate inflammation and initiate repair after diverse types of tissue injury. These macrophages, as well as Th2 cells themselves, induce scar formation by secreting growth factors that stimulate fibroblast proliferation (platelet-derived growth factor), collagen synthesis (IL-13, transforming growth factor-β [TGF-β]), and new blood vessel formation or angiogenesis (fibroblast growth factor). Th2 cytokines also suppress classical macrophage activation and interfere with protective Th1-mediated immune responses to intracellular infections (see Chapter 16). Although the separation of classical and alternative macrophage activation provides a useful context for understanding macrophage heterogeneity, numerous other subpopulations have been described and M1 and M2 macrophages are likely not fixed subsets.

The Th17 Subset

The Th17 subset is primarily involved in recruiting neutrophils and, to a lesser extent, monocytes to sites of infection and inflammation. Some cytokines made by Th17 cells serve to maintain the integrity of epithelial barriers. These actions are critical for destroying bacteria and fungi, microbes that are killed by the phagocytes, and reparing injured epithelial layers, but they also contribute significantly to chronic inflammatory diseases.

Development of Th17 Cells

The development of Th17 cells is stimulated by proinflammatory cytokines produced in response to bacteria and fungi (Fig. 10.11) . Various bacteria and fungi act on DCs and macrophages and stimulate the production of cytokines, including IL-6, IL-1, and IL-23, all of which promote differentiation of CD4$^+$ T cells to the Th17 subset. Engagement of the lectin Dectin-1 on dendritic cells by fungal glucans is a signal for the production of these cytokines. The combination of cytokines that drive Th17 cell development may be produced not only in response to particular microbes, such as fungi, but also when cells infected with various bacteria and fungi undergo apoptosis and are ingested by DCs. Whereas IL-6 and IL-1 stimulate the early steps in Th17 differentiation, IL-23 may be more important for the proliferation and maintenance of differentiated Th17 cells. A surprising aspect of Th17 differentiation is that TGF-β, which is produced by many cell types and is an antiinflammatory cytokine (see Chapter 15), promotes the development of proinflammatory Th17 cells when other mediators of inflammation, such as IL-6 or IL-1, are present. Th17 differentiation is inhibited by IFN-γ and IL-4; therefore, strong Th1 and Th2 responses tend to suppress Th17 development.

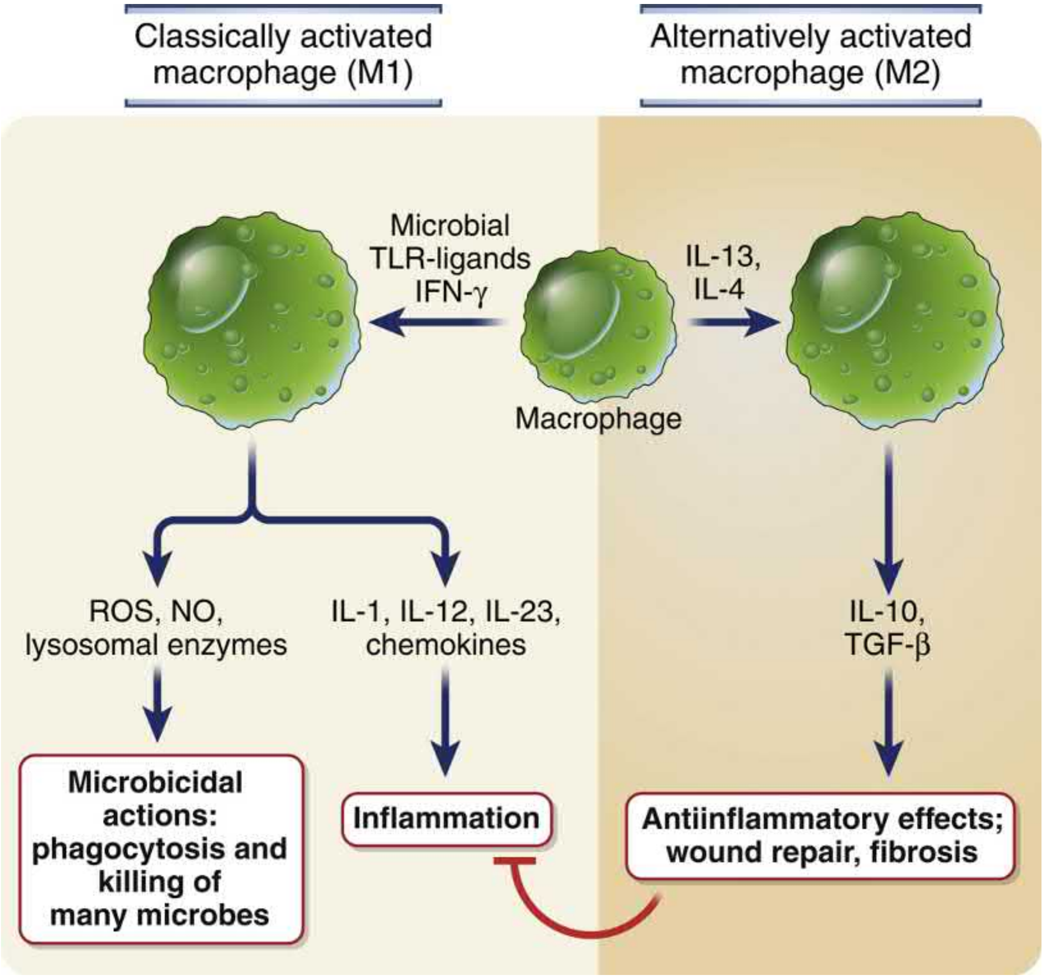

Classically activated macrophage (M1)
Alternatively activated macrophage (M2)
Microbial TLR-ligands IFN-γ
IL-13, IL-4
Macrophage
ROS, NO, lysosomal enzymes
IL-1, IL-12, IL-23, chemokines
IL-10, TGF-β
Microbicidal actions: phagocytosis and killing of many microbes
Inflammation
Antiinflammatory effects; wound repair, fibrosis

FIGURE 10.10 Classical and alternative macrophage activation. Different stimuli activate tissue macrophages to develop into functionally distinct populations. Classically activated macrophages are induced by microbial products and cytokines, particularly interferon-γ *(IFN-γ),* and are microbicidal and involved in potentially harmful inflammation. Alternatively activated macrophages are induced by interleukin-4 *(IL-4)* and IL-13 produced by Th2 cells and other leukocytes and function to control inflammation and to promote tissue repair and fibrosis. Some evidence suggests that M2 macrophages comprise subpopulations, some of which are mainly antiinflammatory and others are responsible for

tissue repair. *NO,* Nitric oxide; *ROS,* reactive oxygen species; *TGF-β,* transforming growth factor-β; *TLR,* Toll-like receptor.

The development of Th17 cells is dependent on the transcription factors RORγt and STAT3 (see Fig. 10.11) **.** TGF-β and the inflammatory cytokines, mainly IL-6 and IL-1, work cooperatively to induce the production of RORγt, a transcription factor that is a member of the retinoic acid receptor family. RORγt is a T cell–restricted protein encoded by the *RORC* gene, so sometimes the protein may be called RORc. Inflammatory cytokines, notably IL-6, activate the transcription factor STAT3, which functions with RORγt to drive the Th17 response.

Th17 cells appear to be abundant in mucosal tissues, particularly of the gastrointestinal tract, suggesting that the tissue environment influences the generation of this subset, perhaps by providing high local concentrations of TGF-β and inflammatory cytokines. This observation also suggests that Th17 cells may be especially important in combating intestinal infections and in the development of pathologic intestinal inflammation. The development of Th17 cells in the gastrointestinal tract is dependent on the gut microbiome; in mice, commensal bacteria related to *Clostridium* species are potent inducers of Th17 cells.

Functions of Th17 Cells

Th17 cells combat microbes by recruiting leukocytes, mainly neutrophils, to sites of infection (Fig. 10.12) **.** Phagocytosis by neutrophils is a major defense mechanism against many common bacteria and fungi that can survive outside cells but are killed in the phagolysosomes of neutrophils. Th17 cells play an important role in defense against these infections by bringing neutrophils to the microbes. Most of the functions of Th17 cells in host defense are mediated by IL-17, but other cytokines produced by this subset may also contribute.

Interleukin-17

IL-17 is an unusual cytokine because neither it nor its receptor is homologous to any other known cytokine-receptor pair. The IL-17 family includes six structurally related proteins, of which IL-17A and IL-17F are the most similar, and the immunologic functions of this cytokine family are mediated primarily by IL-17A. IL-17A is produced by Th17 cells as well as ILC3s and some γδ and $CD8^+$ T cells. IL-17 receptors are multimeric and expressed on a wide range of cells (see Chapter 7). They activate NF-κB and other transcription factors.

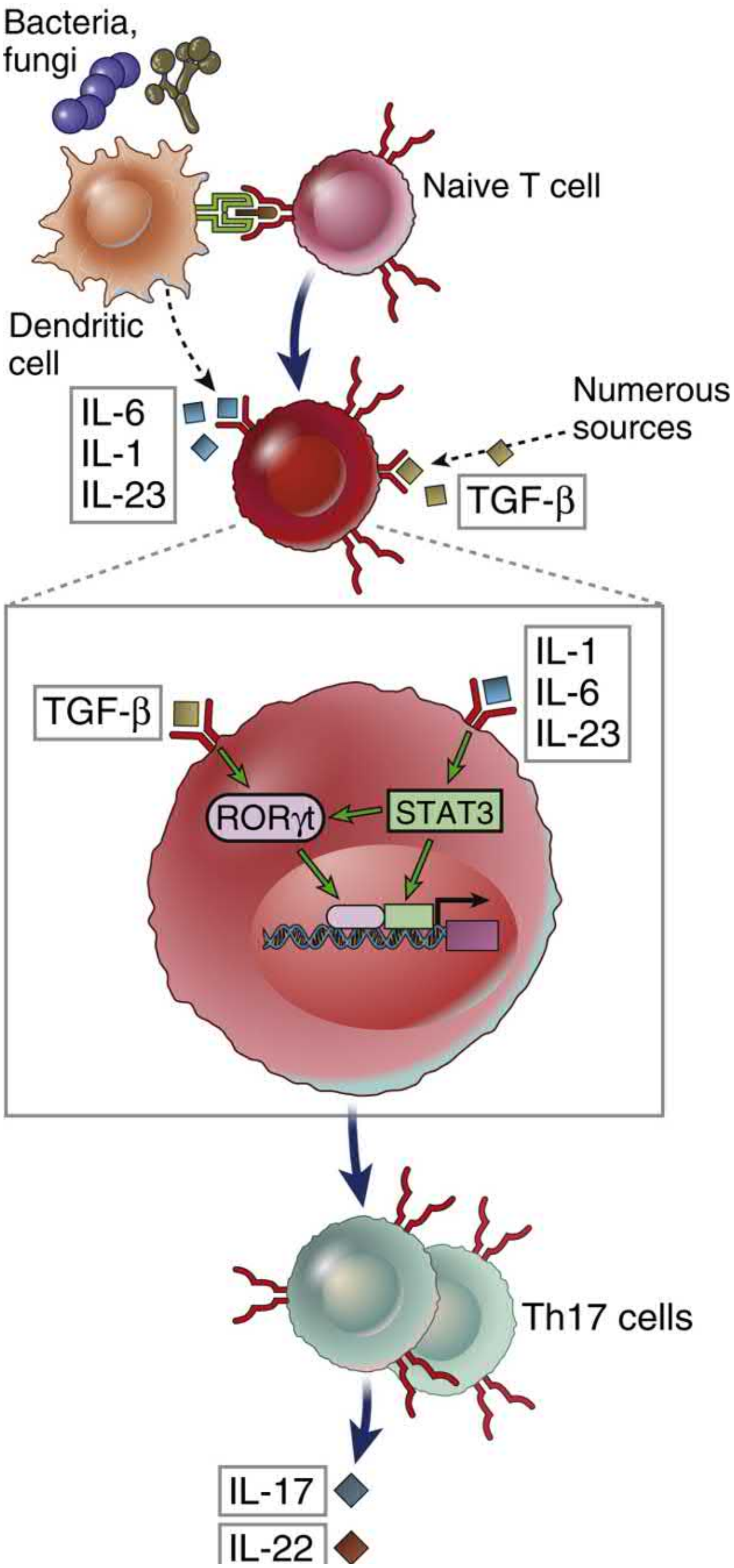

Bacteria,
fungi
Naive T cell
Dendritic
cell
IL-6
IL-1
IL-23
Numerous
sources
TGF-β
TGF-β
IL-1
IL-6
IL-23
RORγt
STAT3
Th17 cells
IL-17
IL-22

FIGURE 10.11 Development of Th17 cells.Interleukin-1 *(IL-1)* and IL-6 produced by antigen-presenting cells (APCs) and transforming growth factor-β *(TGF-β)* produced by various cells activate the transcription factors RORγt and STAT3, which stimulate the differentiation of naive $CD4^+$ T cells to the Th17 subset. IL-23, which is also produced by APCs, especially in response to fungi, stabilizes the Th17 cells. TGF-β may promote Th17 responses indirectly by suppressing Th1 and Th2 cells, both of which inhibit Th17 differentiation (not shown). IL-21 produced by the Th17 cells amplifies this response.

IL-17 is an important link between T cell–mediated adaptive immunity and the acute inflammatory response, which we discussed in Chapter 4 as one of the major reactions of innate immunity. When Th17 cells are activated, these reactions are more severe and prolonged than what is seen in innate immunity, when T cells are not involved.

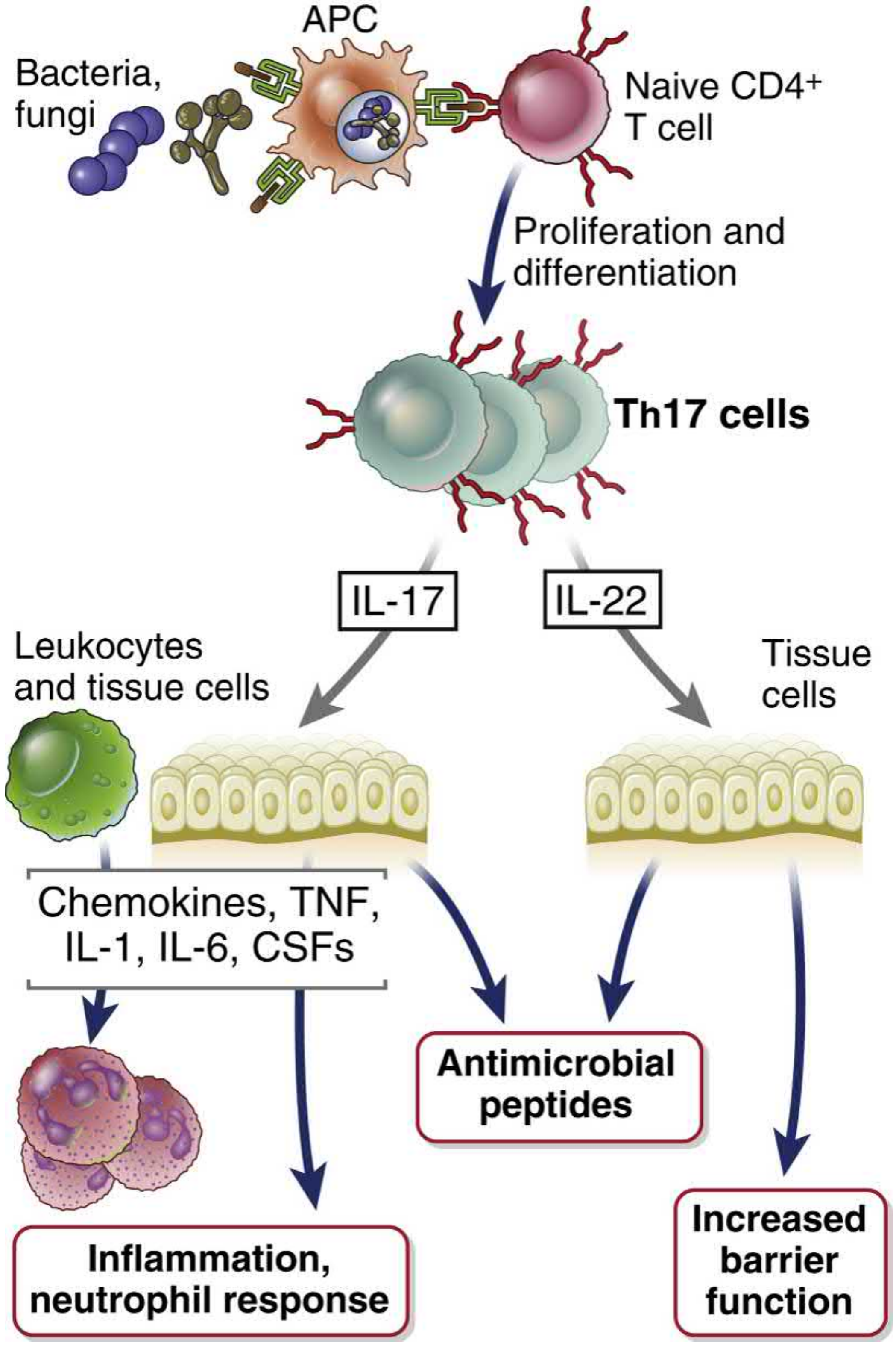

APC
Bacteria, fungi
Naive CD4+ T cell
Proliferation and differentiation
Th17 cells
IL-17
IL-22
Leukocytes and tissue cells
Tissue cells
Chemokines, TNF, IL-1, IL-6, CSFs
Antimicrobial peptides
Inflammation, neutrophil response
Increased barrier function

FIGURE 10.12 Functions of Th17 cells.Cytokines produced by Th17 cells stimulate local production

of chemokines that recruit neutrophils and other leukocytes, increase production of antimicrobial peptides (defensins), and promote epithelial barrier functions. *APC,* Antigen-presenting cell; *CSF,* colony-stimulating factor; *TNF,* tumor necrosing factor.

IL-17 has several important functions in host defense.

- ***IL-17 induces neutrophil-rich inflammation.*** It stimulates the production of chemokines such as IL-8 and other cytokines such as TNF that recruit neutrophils and, to a lesser extent, monocytes to the site of T cell activation. It also enhances neutrophil generation by increasing the production of granulocyte colony-stimulating factor (G-CSF) and the expression of its receptors. Recruited neutrophils ingest and destroy bacteria and fungi.
- ***IL-17 stimulates the production of antimicrobial substances,*** including defensins, from numerous cell types (see Chapter 4).

Other Th17 Cytokines

IL-22 is a member of the type II cytokine family. It is produced by activated T cells, particularly Th17 cells, and by some NK cells and ILCs. The IL-22 receptor is a heterodimer in which one of the chains is also a component of the IL-10 receptor. It signals via JAK1, TYK2, and STAT3. IL-22 is produced by Th17 cells in epithelial tissues, especially of the skin and gastrointestinal tract, and serves to maintain epithelial integrity, mainly by promoting the barrier function of epithelia, by stimulating repair reactions, and by inducing production of antimicrobial peptides. IL-22 also contributes to inflammation, in part by stimulating epithelial production of chemokines, and may therefore be involved in tissue injury in inflammatory diseases.

IL-21 is produced by activated $CD4^+$ T cells, including Th17 cells and Tfh cells. It has a wide variety of effects on B and T cells and NK cells. The IL-21 receptor belongs to the type I cytokine receptor family, consists of a ligand-binding chain and the γc subunit, and activates a JAK-STAT signaling pathway in which STAT3 is especially prominent. An important function of IL-21 is in antibody responses, especially the reactions that occur in germinal centers (see Chapter 12). IL-21 stimulates the generation of Tfh cells and activates B cells in germinal centers. Some of the other reported actions of IL-21 include increasing the proliferation, differentiation, and effector function of $CD8^+$ T cells and NK cells.

Roles of Th17 Cells in Host Defense

The principal function of Th17 cells is to destroy extracellular bacteria and fungi, mainly by inducing neutrophilic inflammation (see Fig. 10.12) **.** The recruited neutrophils ingest and kill extracellular microbes. The importance of this role of Th17 cells is illustrated by the inherited disease called **Job's syndrome** (or hyper-IgE syndrome), which is caused by mutations in STAT3 resulting in defective Th17 development, and is characterized by increased susceptibility to cutaneous fungal and bacterial infections. Patients present with multiple bacterial and fungal abscesses of the skin, resembling the biblical accounts of the punishments visited on Job. Defective Th17 function is also associated with chronic mucocutaneous candidiasis. Patients with the autoimmune disease autoimmune polyendocrine syndrome produce autoantibodies against their own IL-17 and also develop candidiasis (see Chapter 15). Surprisingly, patients with mutations in the *RORC* gene, which encodes RORγt, the canonical transcription factor for Th17 cells, show defects not only in IL-17 production but also in the production of IFN-γ, the classical Th1 cytokine.

Th17 cells contribute to the pathogenesis of many inflammatory diseases. Th17 responses have been associated with psoriasis, inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis. Agents that block the development or functions of Th17 cells are very effective for the treatment of psoriasis and are being tested in other diseases. These antagonists are not beneficial in Crohn's disease, an inflammatory bowel disease, so despite the abundance of Th17 cells in the intestines, the role of these cells in Crohn's disease is uncertain. In some chronic inflammatory diseases, both IL-17 and IFN-γ contribute to the disease. These two cytokines may be produced by a subset of Th17 cells that are thought to be highly pathogenic or by a mixture of Th17 and Th1 cells in the lesions.

Th17 cells help to maintain the integrity of epithelial barriers, such as in the intestinal tract. This function is partly because these T cells limit the entry of infectious microbes through the barriers by stimulating local production of antimicrobial peptides and partly because IL-22 promotes the regeneration of epithelia. It is possible that different populations of Th17 cells are involved in this protective function and in the pathologic reactions caused by this subset.

Functions of Other HELPER T Cell Subsets

Although Th1, Th2, and Th17 subsets are the best defined effector cells of the $CD4^+$ lineage, several other populations of cytokine-producing T cells have been described. Some of these are $CD4^+$ T cells, and others do not belong to either the CD4 or the CD8 lineage.

Other Subsets of $CD4^+$ Effector T Cells

Other subsets of $CD4^+$ T cells that produce various cytokines have been described, but their importance is less clear than that of Th1, Th2, and Th17 cells.

- ***Th9 cells.*** Activation of $CD4^+$ T cells in the presence of the cytokines TGF-β and IL-4 generates a population of effector cells that produce mainly IL-9 and are therefore called Th9 cells. Th9 cells are abundant in the skin and mucosal tissues. IL-9 activates mast cells and has many other reported activities. Th9 cells have been implicated in allergic diseases, including atopic dermatitis and asthma, in defense against parasites, and in numerous other reactions.

- ***Th22 cells.*** Th22 cells were defined as CD4$^+$ T cells that produce IL-22 (a known cytokine of Th17 cells) but not IL-17. The functions of IL-22 were discussed earlier.

The roles of these subsets in host defense or immunologic diseases are not established, in part because it has not been possible to selectively eliminate any of these subsets or their products. Furthermore, a unique transcriptional signature of these cells has not been as well defined, and the possibility exists that that they are transient states in the differentiation of the more established subsets.

Other Cytokine-Producing T Cells

In addition to CD4$^+$ and CD8$^+$ T cells, there are smaller populations of T cells that have distinct features and may serve specialized functions in host defense. The best defined of these lymphocytes are γδ T cells, natural killer T (NKT) cells, and mucosa-associated invariant T (MAIT) cells. All three of these cell types have common characteristics that distinguish them from CD4$^+$ and CD8$^+$ T cells. They recognize a limited number but a wide variety of antigens, many of which are not peptides, and these antigens are not displayed by class I and class II MHC molecules on APCs. The antigen receptors of γδ T cells, NKT cells, and MAIT cells have limited diversity, suggesting that all three cell types may have evolved to recognize a small group of microbial antigens. It is also possible that these cells mainly respond not to particular antigens but to cytokines produced at sites of infection and tissue damage. Because of these features, these T cell populations are often said to be at the crossroads of innate and adaptive immunity. All three cell types are abundant in epithelial tissues, such as the gastrointestinal tract. Their functions may include the following:

- Early defense against microbes encountered at epithelia, before adaptive immune responses have developed
- Surveillance against stressed cells, such as cells that have undergone DNA damage or are infected, and elimination of these cells
- Production of cytokines that influence later adaptive immune responses

γδ T Cells

The antigen receptor of MHC-restricted $CD4^+$ and $CD8^+$ T lymphocytes is a heterodimer composed of α and β chains (see Chapter 7). There is a second type of clonally distributed receptor composed of heterodimers of γ and δ chains, which are homologous to the α and β chains of the TCRs found on $CD4^+$ and $CD8^+$ T lymphocytes. T cells expressing the γδ TCR represent a lineage distinct from the more numerous αβ-expressing T cells. The percentages of γδ T cells vary widely in different tissues and species, but overall, less than 5% of all T cells express this form of TCR. The γδ heterodimer associates with the CD3 and ζ proteins in the same way as TCR αβ heterodimers do, and TCR-induced signaling events typical of αβ-expressing T cells are also observed in γδ T cells. Although in theory the potential diversity of the γδ TCR is even greater than the diversity of the αβ TCR, in reality only a limited number of γ and δ V regions are expressed, and there is little or no junctional diversity.

Different populations of γδ T cells may develop at distinct times during ontogeny, contain different V regions in their antigen receptors, reside in different tissues, and have a limited capacity to recirculate among these tissues. In mice, many skin γδ T cells develop in neonatal life and express one particular TCR with essentially no variability in the V region, whereas many of the γδ T cells in the vagina, uterus, and tongue appear later and express another TCR with a different V region. The limited diversity of the γδ TCRs in many tissues suggests that the antigens recognized by these receptors may be conserved among cell types or microbes that are commonly encountered in these tissues. One intriguing feature of γδ T cells is their abundance in epithelial tissues of certain species. For example, more than 50% of lymphocytes in the small bowel mucosa of mice and chickens, called intraepithelial lymphocytes, are γδ T cells. In mouse skin, many of the intraepidermal T cells express the γδ receptor. Equivalent cell populations are not as abundant in humans; only approximately 10% of human intestinal intraepithelial T cells express the γδ TCR. γδ T cells in lymphoid organs express more diverse TCRs than the epithelial γδ cells.

γδ T cells do not recognize MHC-associated peptide antigens and are not MHC restricted. Some γδ T cell clones recognize small phosphorylated molecules, alkyl amines, or lipids that are commonly found in mycobacteria and other microbes and that may be presented by nonclassical class I MHC–like molecules. Other γδ T cells recognize protein or nonprotein antigens that do not require processing or any particular type of APCs for their presentation. Many γδ T cells are triggered by microbial heat shock proteins. A working hypothesis for the specificity of γδ T cells is that they may recognize antigens that are frequently encountered at epithelial boundaries between the host and the external environment.

A number of biologic activities have been ascribed to γδ T cells, including secretion of cytokines and killing of infected cells, but the function of these cells and their contribution to normal immune responses remain poorly understood. It has been postulated that this subset of T cells may provide protection against microbes at epithelia before the recruitment and activation of antigen-specific αβ T cells. However, mice lacking γδ T cells, created by deletion of the γ or δ TCR gene, have little or no immunodeficiency and only a modest increase in susceptibility to infections by some intracellular bacteria. These cells also may be involved in inflammatory diseases. For instance, in the inflammatory skin disease psoriasis, γδ T cells appear to be among the earliest IL-17–producing cells in lesions. It is not known if this is the case in other inflammatory disorders or what the γδ cells are recognizing or how much they are contributing to the development of the disease.

Natural Killer T (NKT) Cells

A small population of T cells, called NKT cells, expresses markers such as CD56 that are found on NK cells. The TCR α chains expressed by a subset of NKT cells have limited diversity, and in humans these cells are characterized by a TCR α chain with a V region that is encoded by a rearranged Vα24-Jα18 gene segment, with little or no junctional diversity, associated with a TCR β chain that uses one of three Vβ gene segments. Because of this limited diversity, these cells are also called invariant NKT (iNKT) cells. Other NKT cells exist that have quite diverse antigen receptors. All NKT cell TCRs recognize lipids that are bound to class I MHC–like molecules called CD1 molecules. NKT cells and other lipid antigen–specific T cells are capable of rapidly producing cytokines, such as IL-4 and IFN-γ, after activation, and they may help marginal zone B cells to produce antibodies against lipid antigens. NKT cells may mediate protective innate immune responses against some pathogens, such as mycobacteria (which have lipid-rich cell walls), and iNKT cells may even regulate adaptive immune responses primarily by secreting cytokines. However, the roles of these cells in protective immunity or disease are unclear.

Mucosa-Associated Invariant T (MAIT) Cells

MAIT cells are another subset of T cells that express an invariant αβ TCR that uses a rearranged Vα7.2-Jα33 gene segment. MAIT cells recognize fungal and bacterial metabolites of the riboflavin synthesis pathway, presented by a nonpolymorphic class I MHC–like molecule called MR1 (MHC class I–related protein 1). Most MAIT cells are CD8$^+$ and can be activated either by MR1-restricted presentation of microbial riboflavin derivatives or directly by cytokines, including IL-12 and IL-18. The effector functions of

MAIT cells include secretion of inflammatory cytokine such as IFN-γ and TNF and cytotoxicity against infected cells. MAIT cells are found in the blood, the gastrointestinal tract, and the liver; they account for about 50% of all T cells in the human liver. Given their abundance in the liver, it is possible that they represent an important barrier to gut flora that have breached the intestinal epithelial barrier and entered the blood, because blood draining the gut first enters the liver through the portal circulation.

Having concluded our discussion of the functions of CD4$^+$ effector T cells and some less common T cell populations, in Chapter 11 we will consider effector cells of the CD8$^+$ lineage, whose major roles are in defense against viral infections.

Summary

- Cell-mediated immunity is the adaptive immune response stimulated by microbes inside host cells. It is mediated by T lymphocytes and can be transferred from immunized to naive individuals by T cells and not by antibodies.
- Naive CD4$^+$ T lymphocytes may differentiate into different types of specialized effector T cells, including: Th1 cells that secrete interferon-γ (IFN-γ), which mediate defense against intracellular microbes; Th2 cells that secrete interleukin-4 (IL-4) and IL-5, which favor IgE- and eosinophil/mast cell–mediated immune reactions against helminths; or Th17 cells, which promote inflammation and mediate defense against extracellular fungi and bacteria.
- The differentiation of naive CD4$^+$ T cells into subsets of helper T cells is induced by cytokines produced by antigen-presenting cells, by the T cells themselves, and by other cells. The differentiation program is governed by transcription factors that promote cytokine gene expression in the T cells and epigenetic changes in cytokine gene loci, which may be associated with stable commitment to a particular subset. Each subset produces cytokines that

increase its own development and inhibit the development of the other subsets, thus leading to increasing polarization of the response.

- Th1 cells recognize antigens of microbes that have been ingested by phagocytes and activate the phagocytes to kill the microbes. The activation of macrophages by Th1 cells is mediated by IFN-γ and CD40L-CD40 interactions. Activated macrophages kill phagocytosed microbes ingested into phagolysosomes by the actions of reactive oxygen and nitrogen species and enzymes (called classical macrophage activation). Activated macrophages also stimulate inflammation and can damage tissues.
- Th2 cells recognize antigens produced by helminths and other microbes, as well as environmental antigens associated with allergies. IL-4 promotes B cell isotype switching and production of IgE, which may cause mast cell degranulation and inflammation. IL-5 secreted by activated Th2 cells activates eosinophils to release granule contents that destroy helminths but may also damage host tissues. IL-4 and IL-13 together provide protection at epithelial barriers and induce an alternative form of macrophage activation that generates macrophages that control inflammation and mediate tissue repair and fibrosis.
- Th17 cells stimulate neutrophil-rich inflammatory responses that eradicate extracellular bacteria and fungi and maintain the integrity of epithelia. Th17 cells also may be important in mediating tissue damage in autoimmune diseases.
- γδ T cells, natural killer T cells, and mucosa-associated invariant T cells are T cells that express receptors of limited diversity and recognize various antigens without a requirement for major histocompatibility complex–associated presentation. These cells produce cytokines and may contribute to host defense and inflammatory diseases.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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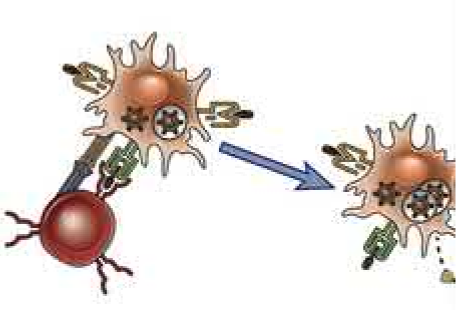
Chapter 11: Differentiation and Functions of CD8$^+$ Effector T Cells



Differentiation of CD8$^+$ T Cells Into Cytotoxic T Lymphocytes,
  Nature of Antigen and Antigen-Presenting Cells for Activation of CD8$^+$ T Lymphocytes,
  Role of Costimulation and Helper T Cells,
  Role of Cytokines and Transcription Factors,
  Inhibition of CD8$^+$ T Cell Responses: T Cell Exhaustion,

Effector Functions of CD8$^+$ Cytotoxic T Lymphocytes,
  Mechanisms of CTL-Mediated Cytotoxicity,
  CD8$^+$ T Cell Memory,

Cytokine Production by CD8$^+$ Effector T Cells,

Roles Of CD8$^+$ Cytotoxic T Lymphocytes In Host Defense,

Summary,

Viruses have evolved to use various cell surface molecules to gain entry into host cells and to use the host cell's genetic and protein synthetic machinery to replicate and to disseminate from one cell to another. Viruses can infect and replicate in a wide variety of cell types. If the infected cells are incapable of destroying the pathogens, one way to eradicate the infection is to kill the infected cell. Viruses are obligate intracellular microbes; therefore, killing the infected cells cripples the ability of the virus to survive in the host. In the adaptive immune system, this function of killing cells harboring viruses is mediated by **cytotoxic T lymphocytes (CTLs),** the effector cells of the CD8$^+$ lineage (see Fig. 10.1B). The same mechanism is used to eliminate phagocytes containing ingested bacteria that escape from phagosomes into the cytosol and are no longer susceptible to the killing activity of the phagocytes. In innate immune reactions, the same function of killing infected cells is mediated by natural killer (NK) cells (see Chapter 4).

In addition to their role in defense against microbes, another important function of CD8$^+$ CTLs is the eradication of tumors. CTLs also play critical roles in the acute rejection of organ allografts.

CTLs not only kill infected and tumor cells, but they also produce the cytokine interferon-γ (IFN-γ), which activates macrophages to destroy ingested microbes and promotes immune defense against many cancers. This activity of CD8$^+$ T cells complements production of the same cytokine by Th1 cells (see Chapter 10). Much of the focus of this chapter is on the specialized cytotoxic function of CD8$^+$ T cells.

In Chapter 6, we discussed the nature of the antigens that are recognized by CD8$^+$ T cells. CD8$^+$ T cells recognize peptides displayed by class I major histocompatibility complex (MHC) molecules, and are said to be class I MHC-restricted. We discussed the early steps of activation of T cells in Chapter 9. There we mentioned some of the features of the responses of CD8$^+$ cells, including their remarkable clonal expansion following activation by antigen and other signals. In this chapter, we will describe how naive CD8$^+$ cells, which lack killing ability, differentiate into functional CTLs and how they kill other cells, then discuss the roles of CTLs in host defense.

Differentiation of CD8$^+$ T Cells Into Cytotoxic T Lymphocytes

Differentiation of CD8 $^+$ ***T cells into effector CTLs involves acquisition of the machinery to kill infected or tumor cells.*** The cell that is killed by CTLs is commonly called the target cell. Naive CD8$^+$ cells recognize antigens but are incapable of killing target cells and need to proliferate and differentiate to generate a sufficiently large pool of functional CTLs to destroy the source of the antigen. Within the cytoplasm of differentiated CTLs are numerous modified lysosomes (called granules) that contain proteins, including perforin and granzymes, whose function is to kill target cells (described later).

The activation of naive CD8$^+$ T cells requires antigen recognition and second signals and proceeds in steps similar to those for CD4$^+$ T cell responses (Fig. 11.1). However, the activation of naive CD8$^+$ T cells has two unique features: it is often dependent on the cross-presentation pathway of antigen presentation by a specialized subset of dendritic cells (DCs), and it may also require help from CD4$^+$ T cells.

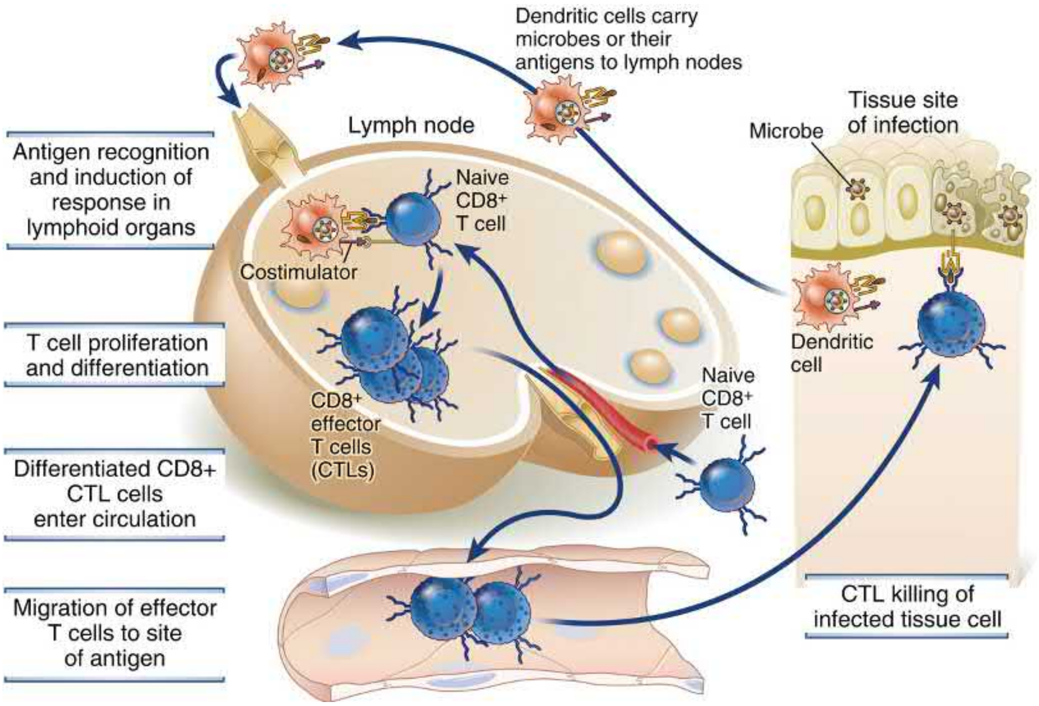

Dendritic cells carry microbes or their antigens to lymph nodes
Tissue site of infection
Microbe
Lymph node
Antigen recognition and induction of response in lymphoid organs
Naive CD8+ T cell
Costimulator
T cell proliferation and differentiation
CD8+ effector T cells (CTLs)
Dendritic cell
Naive CD8+ T cell
Differentiated CD8+ CTL cells enter circulation
Migration of effector T cells to site of antigen
CTL killing of infected tissue cell

FIGURE 11.1 Induction and effector phases of CD8$^+$ T cell responses.Naive CD8$^+$ T cells recognize antigens presented by dendritic cells in secondary lymphoid organs and are stimulated to proliferate and differentiate into effector cells (cytotoxic T lymphocytes *[CTLs]*) and memory cells. The CTLs migrate to tissues at sites of infection, tumor growth, or graft rejection, where they recognize the antigen and respond by killing the cells where the antigen is produced.

Nature of Antigen and Antigen-Presenting Cells for Activation of CD8$^+$ T Lymphocytes

The activation of naive CD8$^+$ T cells, like that of naive CD4$^+$ T cells, is best initiated by antigens presented by DCs. This is primarily because naive T and B cells home to secondary lymphoid organs (and not to nonlymphoid sites of infection, injury, or tumor formation) and tissue-resident DCs capture the antigens of microbes (or tumors) in nonlymphoid tissues and take the antigens to the secondary lymphoid organs through which naive T cells circulate. The antigens that are presented to CD8$^+$ T cells have to be located in the cytosol of the antigen-presenting cells (APCs) because only cytosolic proteins are processed into peptides by proteasomes and delivered to the endoplasmic reticulum to be displayed by class I MHC molecules (see Chapter 6). However, viruses infect specific cell types, and tumors arise from various cell types, and in both instances the cells producing the viral or tumor antigen are usually not DCs. Specialized DCs (especially the classical DC1 [cDC1] subset) ingest infected cells, tumor cells, or proteins produced in these cells, transfer the protein antigens into the cytosol, and process the antigens to enter the class I MHC antigen presentation pathway for recognition by CD8$^+$ T cells. This process of **cross-presentation** is the essential first step in the activation of naive CD8$^+$ T cells.

Role of Costimulation and Helper T Cells

The activation of naive CD8$^+$ T cells, like that of CD4$^+$ cells, requires stimuli in addition to antigen. In viral infections in which the virus induces strong innate immune responses, APCs express costimulators such as B7 molecules, which engage CD28 on the naive T cells and provide the necessary second signals (see Chapter 9). However, in many viral infections, such as those by latent viruses, and in many tumors and organ transplants, the innate response is relatively weak because these viruses, tumors, and transplanted tissues produce few or none of the molecules that activate innate immune receptors. In these situations, the second signals may be provided by CD4$^+$ helper T cells.

CD4$^+$ helper T cells promote naive CD8$^+$ T cell activation by several mechanisms (Fig. 11.2). Helper T cells can secrete cytokines that stimulate the differentiation of CD8$^+$ T cells. The nature of these cytokines is discussed in the section that follows. Activated helper T cells express CD40 ligand (CD40L), which may bind to

CD40 on antigen-loaded DCs. This interaction activates the APCs to make them more efficient at stimulating the differentiation of CD8$^+$ T cells, in part by increasing the expression of costimulators. This process has been termed licensing of the APCs.

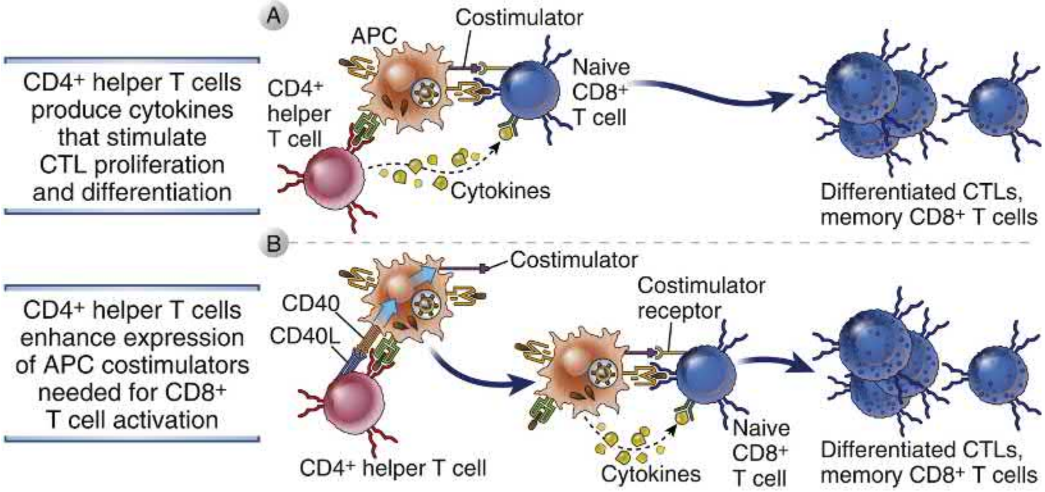

A
CD4+ helper T cells produce cytokines that stimulate CTL proliferation and differentiation
APC
Costimulator
CD4+ helper T cell
Naive CD8+ T cell
Cytokines
Differentiated CTLs, memory CD8+ T cells
B
CD4+ helper T cells enhance expression of APC costimulators needed for CD8+ T cell activation
Costimulator
CD40
CD40L
Costimulator receptor
CD4+ helper T cell
Cytokines
Naive CD8+ T cell
Differentiated CTLs, memory CD8+ T cells

FIGURE 11.2 Role of helper T cells in the differentiation of CD8$^+$ T lymphocytes.CD4$^+$ helper T cells promote the development of CD8$^+$ cytotoxic T lymphocytes *(CTLs)* and memory cells by secreting cytokines that act directly on the CD8$^+$ cells **(A)** or by activating antigen-presenting cells *(APCs)* to become more effective at stimulating the differentiation of the CD8$^+$ T cells, for example, by increasing the expression of costimulators on the APCs **(B).** *CD40L,* CD40 ligand.

The varying importance of CD4$^+$ T cells in the development of CTL responses is illustrated by studies with mice that lack CD4$^+$ T cells. In these mice, some viral infections fail to generate effective CTLs or CD8$^+$ memory cells and are not eradicated, whereas other viruses do stimulate effective CTL responses. A lack of CD4$^+$ T cell helper function accounts for the defects in CTL generation seen in individuals infected with human immunodeficiency virus (HIV), which infects and eliminates only $CD4^+$ T cells. There is also evidence that $CD4^+$ helper cells are more important for the generation of $CD8^+$ memory T cells than for the differentiation of naive $CD8^+$ T cells into effector CTLs.

As we discuss later, once effector cells (differentiated CTLs with the machinery to kill other cells) are produced, they can be activated without any need for costimulation and kill any cell that expresses an antigenic protein in the cytosol and presents a relevant peptide on class I MHC molecules.

Role of Cytokines and Transcription Factors

Several cytokines contribute to the differentiation of $CD8^+$ T cells and the maintenance of effector and memory cells of this lineage.

- Interleukin-2 (IL-2) produced by the $CD8^+$ T cells themselves or by $CD4^+$ helper cells promotes proliferation of the $CD8^+$ T cells and their differentiation into CTLs and memory cells. $CD8^+$ cells express the β and γ chains of the IL-2 receptor and may express high levels of the *α* chain transiently after activation (see Chapter 9).
- IL-12 and type I IFNs have both been shown to stimulate the differentiation of naive $CD8^+$ T cells into effector CTLs. These cytokines may be produced by different DC populations during the innate immune response to viral and some bacterial infections. Recall that the same cytokines are involved in the differentiation of $CD4^+$ T cells into Th1 cells. The cytokines promote development of these two effector populations by stimulating expression of the related transcription factors T-BET (for both Th1 cells and CTLs) and eomesodermin (for CTLs).
- IL-15 is important for the survival of memory $CD8^+$ T cells. It may be produced by many cell types, including DCs. Mice

lacking IL-15 show a significant loss of memory CD8$^+$ T cells.

Three transcription factors that are required for the program of gene expression that occurs during CTL differentiation are T-BET (which we discussed in relationship to Th1 differentiation in Chapter 10); eomesodermin, which is structurally related to T-BET; and BLIMP-1. The optimal expression of these transcription factors depends on IL-2, IL-12 and type I interferons and the JAK-STAT signaling pathways they activate. The cytokines work together to promote the transcriptional program of CTL differentiation. For example, IL-2–induced STAT5 together with IL-12-induced STAT4 are required for high expression of T-BET and BLIMP-1, which stimulate the expression of perforin, granzymes, and some cytokines, especially IFN-γ.

Inhibition of CD8$^+$ T Cell Responses: T Cell Exhaustion

In some chronic viral infections and cancers, CTL effector responses are generated, but they are then gradually extinguished, a phenomenon that is called exhaustion (Fig. 11.3) **.** The term exhaustion has been used to imply that the effector response starts but is shut down (unlike in tolerance, in which lymphocytes fail to develop into effector cells). This phenomenon of exhaustion was first described in a chronic viral infection in mice and was implicated in the prolonged persistence of the virus. Strong and persistent immune responses to chronic infections have the risk of causing significant collateral tissue damage. T cell exhaustion may have evolved as a mechanism to limit the immunopathology associated with chronic infection. The same mechanism likely down-regulates the host response to any chronic or persistent antigenic stimulus.

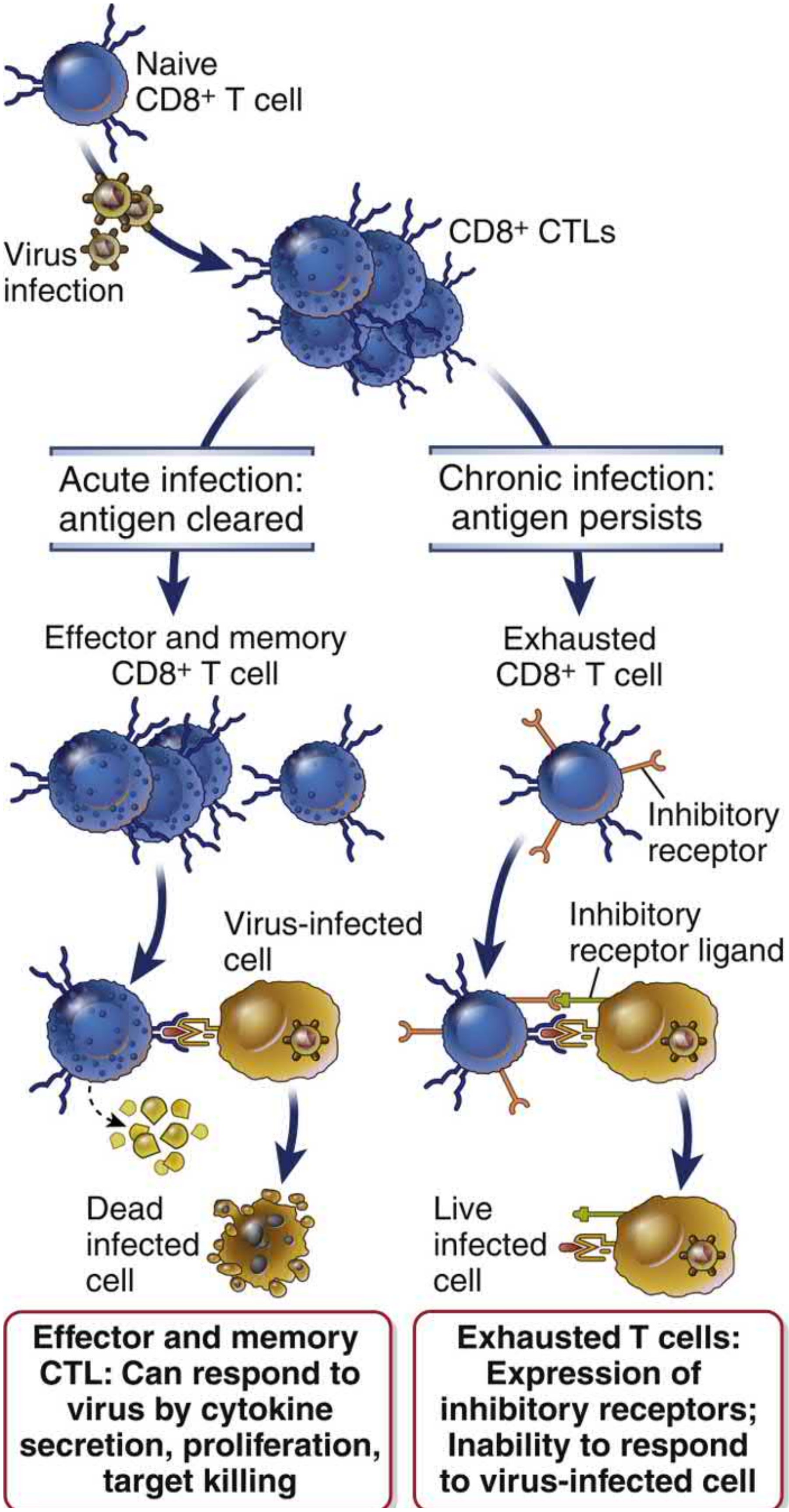

Naive
CD8+ T cell
Virus
infection
CD8+ CTLs
Acute infection:
antigen cleared
Chronic infection:
antigen persists
Effector and memory
CD8+ T cell
Exhausted
CD8+ T cell
Inhibitory
receptor
Virus-infected
cell
Inhibitory
receptor ligand
Dead
infected
cell
Live
infected
cell
Effector and memory CTL: Can respond to virus by cytokine secretion, proliferation, target killing
Exhausted T cells: Expression of inhibitory receptors; Inability to respond to virus-infected cell

FIGURE 11.3 T cell exhaustion.In acute infections, CD8$^+$ T cells differentiate into cytotoxic T lymphocytes *(CTLs)* that eliminate the infected cells. In situations of persistent or chronic antigen exposure, the response of CD8$^+$ T cells is suppressed by the expression and engagement of *PD-1* (programmed cell death protein-1) and other inhibitory receptors.

Repeated stimulation leads to numerous functional defects in the T cells, including decreased proliferative capacity, reduced production of IFN-γ, and poor cytotoxic activity, and the CD8$^+$ cells are thus unable to clear infections or tumors. These defects result from a block in T cell differentiation associated with increased expression of multiple inhibitory receptors in repeatedly stimulated T cells. These inhibitory receptors include PD-1 (programmed cell death protein-1) (see Chapter 9) as well as CTLA-4, TIM-3, LAG-3, and others. An important role for PD-1 as a mediator of exhaustion is shown by the reversal of the exhausted phenotype by anti–PD-1 monoclonal antibodies. Studies in mice show that antigen recognition by memory CD8$^+$ T cells with concurrent PD-1 signaling drives the cells into a permanently disabled (exhausted) phenotype, and PD-1 blockade induces effective responses by allowing activation of the memory cells into functional (non-exhausted) effectors. T cell exhaustion may contribute to the chronicity of some viral infections in humans, such as HIV and hepatitis C virus (HCV), and to the ability of some tumors to evade the immune response (see Chapter 18).

Effector Functions of CD8$^+$ Cytotoxic T Lymphocytes

CD8 $^+$ CTLs eliminate intracellular microbes mainly by killing infected cells (see Fig. 10.1B) . By secreting IFN-γ, CD8$^+$ T cells also contribute to classical macrophage activation in host defense and in hypersensitivity reactions (see Chapter 10). Here we discuss the mechanisms by which differentiated CTLs kill cells harboring microbes; CTLs use the same mechanisms to kill tumors (see Chapter 18).

Mechanisms of CTL-Mediated Cytotoxicity

CTL-mediated killing involves specific recognition of target cells and delivery of proteins into the target that induce cell death. CTLs kill targets that display the same peptide–class I MHC antigen that triggered the proliferation and differentiation of naive CD8$^+$ T cells into functional CTLs. CTL killing is highly antigen-specific, and adjacent uninfected cells that do not present that peptide-MHC antigen are not harmed. Killing specificity is achieved because a close region of contact between the CTL and the antigen-expressing target cell, called an immune synapse (see Chapter 7), is established, and the molecules that actually perform the killing are secreted into the synapse and do not diffuse to other nearby cells. The target cells are killed by apoptosis, which is a pathway of cell death that does not induce harmful inflammation (unlike necrosis, which does induce inflammation). Thus, there is no collateral damage of adjacent normal tissues during CTL-mediated killing.

The process of CTL-mediated killing of targets consists of antigen recognition, activation of the CTLs, delivery of the lethal hit that kills the target cells, and release of the CTLs (Fig. 11.4). Each of these steps is controlled by specific molecular interactions.

Recognition of Antigen and Activation of CTLs

The CTL binds and reacts to the target cell by using its antigen receptor, CD8 coreceptor, and adhesion molecules. To be efficiently recognized by CTLs, target cells must express class I MHC molecules displaying a peptide. The MHC molecule with bound peptide serves as the ligand for the T cell receptor (TCR), and the MHC molecule is the ligand for CD8. Signaling by the TCR promotes formation of the specialized immune synapse, with a central region containing TCRs and other signaling molecules and an outer ring of integrins, notably LFA-1 (leukocyte function–associated antigen 1) on the CTL binding to its ligand ICAM-1 (intercellular adhesion molecule 1) on the target cell (Fig. 11.5). An enclosed gap is present within the ring between the membranes of the two cells. Distinct regions of the CTL membrane can be observed by immunofluorescence microscopy within the ring, including a signaling patch, which includes the TCR, CD8, and signaling proteins (such as protein kinase C-θ and the tyrosine kinase LCK); and a secretory region, which appears as a gap on one side of the signaling patch. TCR engagement by antigen results in the initiation of biochemical signals that activate the CTL, leading to the process of cell killing, described in the following section. Cytokines and costimulators provided by DCs, as well as T cell help, which are required for the differentiation of naive CD8$^+$ T cells into CTLs, are not necessary for triggering the effector function of CTLs (i.e., target cell killing).

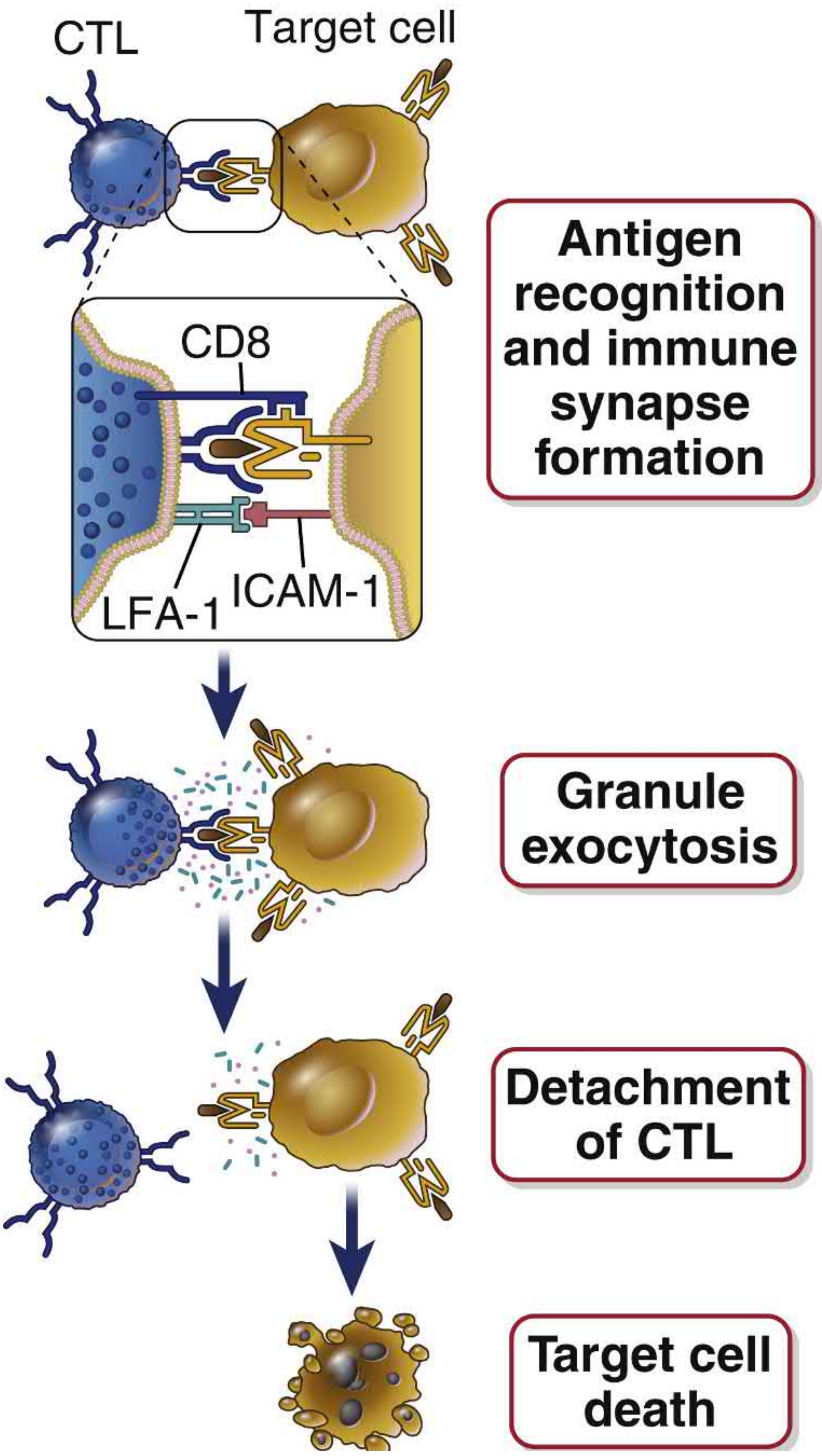

CTL
Target cell
CD8
LFA-1
ICAM-1
Antigen recognition and immune synapse formation
Granule exocytosis
Detachment of CTL
Target cell death

FIGURE 11.4 Steps in cytotoxic T lymphocyte–mediated lysis of target cells.A cytotoxic T lymphocyte *(CTL)* recognizes the antigen-expressing target cell and is activated. Activation results in the release of granule contents from the CTL into the target cell through the area of contact (the immunologic synapse). Granule contents deliver a lethal hit to the target. The CTL may detach and kill other target cells. The formation of conjugates between a CTL and its target and activation of the CTL also require interactions between accessory molecules (leukocyte function–associated antigen 1 *[LFA-1],* CD8) on the CTL and their specific ligands (intercellular adhesion molecule 1 *[ICAM-1]* and class I major histocompatibility complex, respectively) on the target cell (not shown).

In addition to the TCR, $CD8^+$ CTLs express receptors that are also expressed by NK cells, which contribute to both regulation and activation of CTLs. Some of these receptors belong to the KIR (killer immunoglobulin receptor) family, discussed in Chapter 4, and recognize class I MHC molecules on target cells but are not specific for a particular peptide-MHC complex. These KIRs transduce inhibitory signals that may serve to prevent CTLs from killing normal cells. In addition, CTLs express the NKG2D receptor, which recognizes the class I MHC–like molecules MIC-A, MIC-B, and ULBP, which are expressed on stressed (infected or transformed) cells. NKG2D may deliver signals that act together with TCR recognition of antigen to enhance killing activity.

Killing of Target Cells by CTLs

The principal mechanism of CTL-mediated target cell killing is the delivery of cytotoxic proteins stored within cytoplasmic granules (also called secretory lysosomes) to the target cell, thereby triggering

apoptosis of the target cell (Fig. 11.6) **.** Within a few minutes after a CTL's antigen receptor and coreceptor recognize a peptide-MHC complex on the target cell, the CTL granule proteins enter the target cell, and death occurs during the following 2 to 6 hours, even if the CTL detaches. Thus, the CTL is said to deliver a lethal hit to the target cell. When the CTL recognizes antigen, TCR signals lead to actin cytoskeleton reorganization. In this process, the microtubule organizing center of the CTL moves to the area of the cytoplasm near the contact with the target cell. The cytoplasmic granules of the CTL are transported along microtubules and become concentrated in the region of the synapse, and the granule membrane fuses with the plasma membrane at the region of the synapse. Membrane fusion results in exocytosis of the CTL's granule contents into the confined space within the synaptic ring, between the plasma membranes of the CTL and target cell.

The major proteins in the granules of CTLs (and NK cells) that are required for cytotoxicity are granzymes and perforin. In human T cells, there are five different granzymes, namely A, B, H, K, and M; of these, granzymes A, B, H and K are expressed at high levels in $CD8^+$ CTLs. All granzymes are serine proteases; granzyme B cleaves proteins after aspartate residues. It can cleave and thereby activate caspases, which induce apoptosis. The granules also contain a sulfated proteoglycan, serglycin, which holds granzymes and perforin in the granules in an inactive state.

Perforin is a membrane-perturbing molecule that is homologous to the C9 complement protein. Its main function is to facilitate delivery of the granzymes into the cytosol of the target cell. How this is accomplished is still not well understood. Perforin can polymerize and form aqueous pores in the cholesterol-containing target cell plasma membrane, but these pores may not be of sufficient size to allow granzymes to enter. According to one model, complexes of granzyme B, perforin, and serglycin are discharged from the CTL onto the target cell, and perforin insertion into the target cell membrane elicits a membrane repair process, which leads to internalization of both the perforin and granzymes into endosomes. Perforin may then act on the endosomal membrane to facilitate the release of the granzymes into the target cell cytosol. Once in the cytosol, the granzymes cleave various substrates, including caspases, and initiate apoptotic death of the cell. For example, granzyme B cleaves and activates caspase-3 as well as the BCL-2 family member BID, which triggers the mitochondrial pathway of apoptosis (see Fig. 15.10). Another protein found in human CTL (and NK cell) granules, called granulysin, can damage cholesterol-poor membranes, typical of bacteria but not mammalian cells. This leads to delivery of granzymes into the microbes and induction of reactive oxygen species, resulting in killing of intracellular microbes.

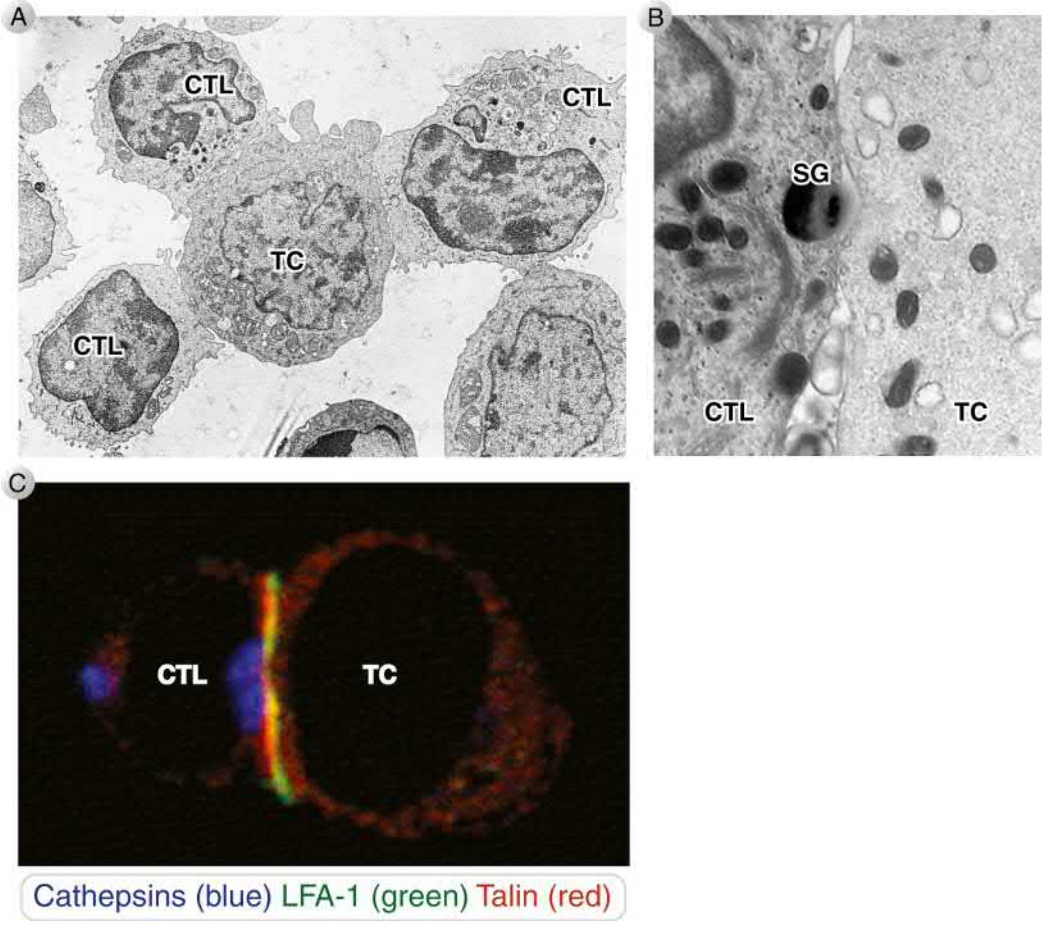


FIGURE 11.5 Formation of conjugates between cytotoxic T lymphocytes and a target cell. **A,** Electron micrograph of three cytotoxic T lymphocytes *(CTLs)* from a cloned cell line specific for the human major histocompatibility complex molecule human leukocyte antigen-A2 *(HLA-A2)* binding to an HLA-A2–expressing target cell *(TC)* within 1 minute after the CTLs and targets are mixed. Note that in the CTL on the *upper left,* the granules have been redistributed toward the target cell. **B,** Electron micrograph of the point of membrane contact between a CTL *(left)* and target cell *(right)*. Two CTL granules (secretory granules *[SG]*) are near the synapse. Several mitochondria are also visible. **C,** Confocal fluorescence micrograph of an immune synapse

between a CTL *(left)* and target cell *(right)* stained with antibodies against cathepsins in a secretory granule *(blue),* leukocyte function–associated antigen 1 *(LFA-1) (green),* and the cytoskeletal protein talin *(red).* The image demonstrates the central location of the secretory granule and the peripheral location of the adhesion molecule LFA-1 and associated cytoskeletal protein talin.

A, courtesy Dr. P. Peters, Netherlands Cancer Institute, Amsterdam; *B,* from Stinchcombe JC, Bossi G, Booth S, Griffiths GM. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity.* 2001;8:751–761; Copyright Cell Press, with permission from Elsevier; *C,* from Stinchcombe JC, Griffiths GM. The role of the secretory immunological synapse in killing by CD8+ CTL. *Semin Immunol.* 2003;15:301–205. Copyright 2003 Elsevier Science Ltd.

CTLs also use a granule-independent mechanism of killing that is mediated by interactions of membrane molecules on the CTLs and target cells. On activation, CTLs express a membrane protein called **FAS ligand (FAS-L)** that binds to the death receptor FAS, which is expressed on many cell types. This interaction also results in activation of caspases and apoptosis of FAS-expressing targets (see Fig. 15.10). Studies with knockout mice lacking perforin, granzyme B, or FAS-L indicate that perforin and granzyme B are the principal mediators of killing by CD8$^+$ CTLs.

After delivering the lethal hit, the CTL is released from its target cell, which usually occurs even before the target cell dies. CTLs themselves are not injured during target cell killing, and two mechanisms have been proposed for CTL protection. First, CTLs express a serine protease inhibitor in the cytosol called Spi6 that can antagonize granzymes, including granzyme B. Second, CTL granules contain a proteolytic enzyme called cathepsin B that is delivered to the CTL surface on granule exocytosis, where it degrades errant perforin molecules that come into the vicinity of the CTL membrane. How cathepsin B is presumably preferentially delivered to the surface of CTLs and not of target cells is not known.

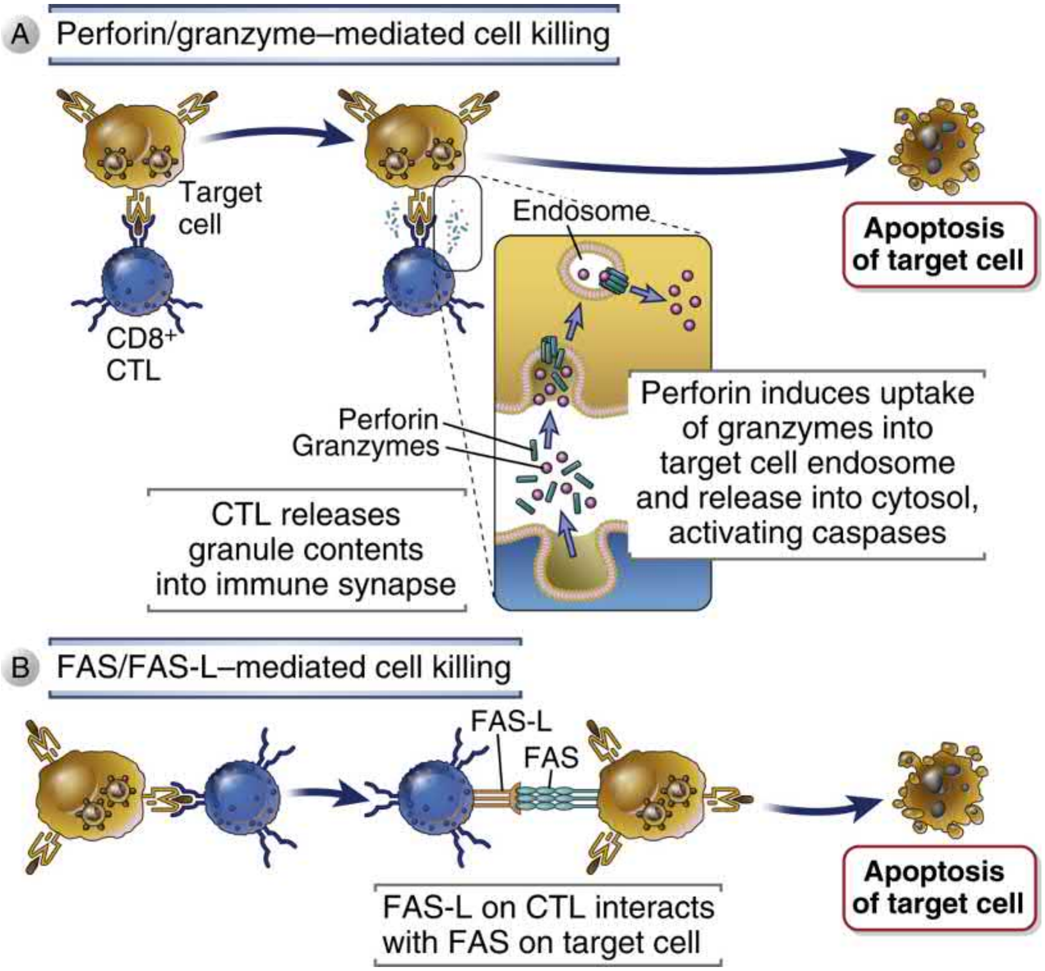

A
Perforin/granzyme–mediated cell killing
Target cell
CD8+ CTL
Endosome
Apoptosis of target cell
Perforin
Granzymes
CTL releases granule contents into immune synapse
Perforin induces uptake of granzymes into target cell endosome and release into cytosol, activating caspases
B
FAS/FAS-L–mediated cell killing
FAS-L
FAS
FAS-L on CTL interacts with FAS on target cell
Apoptosis of target cell

FIGURE 11.6 Mechanisms of cytotoxic T lymphocyte–mediated killing of target cells.Cytotoxic T lymphocytes *(CTLs)* kill target cells by two main mechanisms. **A,** Complexes of perforin and granzymes are released from the CTL by granule exocytosis and enter target cells. The granzymes are delivered into the cytoplasm of the target cells by a perforin-dependent mechanism, and they induce apoptosis. **B,** FasL is expressed on activated CTLs, engages Fas on the surface of target cells, and induces apoptosis.

CD8$^+$ T Cell Memory

Following activation, naive CD8$^+$ T cells differentiate not only into functional CTLs but also into long-lived memory cells. Help from CD4$^+$ T cells may be especially important for the generation of these memory cells. The memory CD8$^+$ T cells are functionally inactive and have to be restimulated by antigen to develop into active effector CTLs. The general principles of CD8$^+$ T cell memory generation are similar to those for all T cells, discussed in Chapter 9. Tissue-resident memory T (T_{RM}) cells, which remain in nonlymphoid tissues such as the skin for long periods without recirculating, were described in Chapter 9. Many of these T_{RM} cells are CD8$^+$ T cells.

Cytokine Production by CD8$^+$ Effector T Cells

CD8$^+$ T cells produce the macrophage-activating cytokine IFN-γ. In fact, the secretion of IFN-γ in response to specific peptides is a sensitive assay for the frequency of antigen-specific CD8$^+$ T cells in a population of lymphocytes. It is likely that both CD4$^+$ Th1 cells and CD8$^+$ T cells contribute to IFN-γ–induced phagocytic clearance of ingested microbes. CD8$^+$ cells may also play a role in some cytokine-induced inflammatory reactions, such as contact sensitivity skin reactions induced by environmental chemicals, where IFN-γ–producing CD8$^+$ T cells often arrive earlier than and outnumber CD4$^+$ T cells. IL-17 producing CD8$^+$ T cells are abundant in some chronic inflammatory diseases of the skin, such as psoriasis.

Roles of CD8$^+$ Cytotoxic T Lymphocytes in Host Defense

In infections by intracellular microbes, the killing activity of CTLs is important for eradication of the reservoir of infection (see Fig. 10.1B). This is particularly important in two types of situations when cells cannot destroy microbes that infect them. First, most viruses live and replicate in cells that lack the phagosome/lysosome machinery for destroying microbes (such as hepatitis viruses in liver cells). Second, even in phagocytes, some microbes escape from vesicles and live in the cytosol, where microbicidal mechanisms are ineffective because these mechanisms are largely restricted to vesicles (to protect the host cells from damage). Bacteria such as *Mycobacterium tuberculosis* and *Listeria monocytogenes* are examples of microbes that escape from vesicles and enter the cytosol of infected cells. Such viral and bacterial infections can be eliminated only by destroying the infected cells, and in adaptive immune responses, $CD8^+$ CTLs are the principal mechanism for killing infected cells (see Chapter 16). In addition, the caspases that are activated in target cells by granzymes and FAS-L cleave many substrates and activate enzymes that degrade DNA, but they do not distinguish between host and microbial molecules. Therefore, by activating nucleases in target cells, CTLs can initiate the destruction of microbial DNA as well as the target cell genome, thereby eliminating potentially infectious DNA. The massive expansion of $CD8^+$ T cells that follows infections (see Fig. 9.12) provides a large pool of CTLs to combat these infections. Defects in the development and activity of CTLs result in increased susceptibility to viral and some bacterial infections and reactivation of latent virus infections (such as infection by the Epstein-Barr virus) that are normally kept in check by virus-specific CTLs.

Destruction of infected cells by CTLs is a cause of tissue injury in some infectious diseases. For instance, in infection by hepatitis B and C viruses, the infected liver cells are killed by the host CTL (and NK cell) response and not by the viruses. These viruses are not highly cytopathic, but the host senses and reacts against the infectious microbe and is not able to distinguish microbes that are intrinsically harmful or relatively harmless (see Chapter 19). CTLs may contribute to the immunopathology associated with many other common viral infections, such as influenza.

CTLs are also important mediators of tumor immunity, as discussed in Chapter 18. In addition to their protective roles, $CD8^+$ CTLs contribute to tissue destruction in some autoimmune diseases (see Chapter 19) and to the rejection of tissue grafts (see Chapter 17).

Inherited mutations that interfere with CTL function, such as mutations in perforin and in genes encoding proteins involved in granule exocytosis, are associated with the rare familial form of a disease called **hemophagocytic lymphohistiocytosis (HLH),** one of a group of disorders called **macrophage activation syndromes.** In these disorders, CTLs that are activated by viral antigen secrete IFN-γ, but they do not kill the virus-infected cells because they cannot deliver the lethal hit. Thus, there is persistence of viral antigen, chronic IFN-γ production from the $CD8^+$ T cells, and excessive macrophage activation by the IFN-γ. The severe and prolonged macrophage activation underlies the manifestations of the disease, including enlargement of the spleen caused by increased numbers of activated macrophages (lymphohistiocytosis) that phagocytose and destroy normal red blood cells (hemophagocytosis) (see Chapter 21). An antibody that blocks IFN-γ has been approved for the treatment of macrophage activation syndrome.

Summary

- T cells of the $CD8^+$ subset proliferate and differentiate into cytotoxic T lymphocytes (CTLs), which express cytotoxic granules and can kill infected cells.
- The differentiation of $CD8^+$ T cells into functional CTLs and memory cells requires recognition of antigen presented by dendritic cells, signals from $CD4^+$ helper T cells in some situations, costimulation, and cytokines. Differentiation to CTLs involves the acquisition of the machinery to kill target cells and is driven by various transcription factors.

- In some situations of chronic antigen exposure (such as tumors and chronic viral infections), $CD8^+$ T cells initiate a response but begin to express inhibitory receptors that suppress the response, a process called exhaustion.
- $CD8^+$ CTLs kill cells that express peptides derived from cytosolic antigens (e.g., viral antigens) that are presented in association with class I MHC molecules. CTL-mediated killing is mainly the result of exocytosis of secretory granules that contain granzymes and perforin. Perforin facilitates granzyme entry into the cytoplasm of target cells, and granzymes initiate the process of apoptosis. Another granule protein, granulysin, destroys some intracellular bacteria and fungi.
- $CD8^+$ T cells also secrete interferon-γ and thus may participate in defense against phagocytosed microbes and in delayed-type hypersensitivity reactions.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

Activation and Exhaustion of $CD8^+$ T Lymphocytes

∗Barber D.L, Wherry E.J, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* . 2006;439:682–687 *(The demonstration that in chronic infection, $CD8^+$ T cells become "exhausted" and this process can be reversed by blocking the inhibitory receptor PD-1.).*

Blank C.U, Haining W.N, Held W, et al. Defining "T cell exhaustion. *Nat Rev Immunol.* 2019;19:665–674.

∗Butz E.A, Bevan M.J. Massive expansion of antigen-specific $CD8^+$ T cells during an acute virus infection. *Immunity* . 1998;8:167–175; and Murali-

Krishna K, Altman J.D, Suresh M, et al. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* . 1998;8:177–187 *(The demonstration of massive expansion of viral antigen-specific CD8^{+} T cell clones following exposure to the virus.).*

Halle S, Halle O, Forster R. Mechanisms and dynamics of T cell-mediated cytotoxicity in vivo. *Trends Immunol* . 2017;38:432–443.

Hashimoto M, Kamphorst A.O, Im S.J, et al. CD8 T cell exhaustion in chronic infection and cancer: opportunities for interventions. *Annu Rev Med* . 2018;69:301–318.

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McLane L.M, Abdel-Hakeem M.S, Wherry E.J. CD8 T cell exhaustion during chronic viral infection and cancer. *Annu Rev Immunol* . 2019;37:457–495.

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Mechanisms of CTL-Mediated Cytotoxicity

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Dotiwala F, Lieberman J. Granulysin: killer lymphocyte safeguard against microbes. *Curr Opin Immunol* . 2019;60:19–29.

Golstein P, Griffiths G.M. An early history of T cell-mediated cytotoxicity. *Nat Rev Immunol* . 2018;18:527–535.

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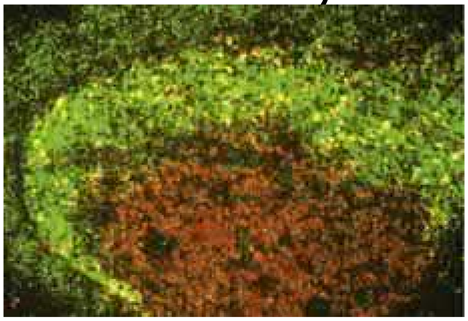
Chapter 12: B Cell Activation and Antibody Production



Overview Of Humoral Immune Responses,

Antigen Recognition and Antigen-Induced B Cell Activation,
    Antigen Capture and Delivery to B Cells,
    Activation of B Cells by Antigens and Other Signals,

Helper T Cell–Dependent Antibody Responses to Protein Antigens,
    The Sequence of Events During T Cell–Dependent Antibody Responses,
    Initial Activation and Migration of Helper B Cells and T Cells,
    Antigen Presentation by B Cells and the Hapten-Carrier Effect,
    Role of CD40L:CD40 Interaction in T-Dependent B Cell Activation,
    Extrafollicular B Cell Activation,

    - T Follicular Helper (Tfh) Cells,
    - The Germinal Center Reaction,
    - Heavy Chain Isotype (Class) Switching,
    - Affinity Maturation: Somatic Mutation of Immunoglobulin Genes and Selection of High-Affinity B Cells,
    - B Cell Differentiation Into Antibody-Secreting Plasma Cells,
    - Generation of Memory B Cells,
    - Role of Transcriptional Regulators in Determining the Fate of Activated B Cells,
- Antibody Responses to T-Independent Antigens,
    - Subsets of B Cells That Respond to T-Independent Antigens,
    - Mechanisms of T-Independent Antibody Responses,
    - Protection Mediated by T-Independent Antibodies,
- Antibody Feedback: Regulation of Humoral Immune Responses by Fc Receptors,
- Summary,

Humoral immunity is mediated by secreted antibodies, which are produced by cells of the B lymphocyte lineage. This chapter describes the molecular and cellular events of the humoral immune response, in particular the stimuli that induce B cell proliferation and differentiation and how these stimuli influence the type of antibody that is produced. The mechanisms by which antibodies eliminate microbes are described in Chapter 13.

Overview of Humoral Immune Responses

The activation of B cells results in their proliferation and their eventual differentiation into antibody-secreting plasma cells and memory cells (Fig. 12.1) **.** Humoral immune responses are initiated by specific B cell recognition of antigen in secondary lymphoid organs. Antigen binds to membrane immunoglobulin M (IgM) and IgD on mature, naive B cells, generating signals required for their proliferation and differentiation into plasma cells. The antibody that is secreted by the plasma cell has essentially the same specificity as the original antibody that served as the antigen receptor on the surface of the naive B cell. A single B cell may, within a week, give rise to as many as 5000 antibody-secreting cells, each of which can secrete about 2000 antibody molecules every second. This cell expansion and the remarkable rate of antibody secretion are needed to keep pace with rapidly dividing microbes.

Antibody responses are T-dependent or T-independent, depending on the nature of the antigen and the involvement of helper T cells (Fig. 12.2) **.** Most responses to protein antigens require T cell help, so these antigens are called T-dependent. The term helper T lymphocyte came from the realization that T cells stimulate, or help, B lymphocytes to produce antibodies. In T-dependent responses, some activated B cells begin to produce antibodies other than IgM; this process is called heavy chain isotype (class) switching (see Fig. 12.1). As the response develops, activated B cells produce antibodies that bind to antigens with increasing affinity, and these B cells progressively dominate the response; this process is called affinity maturation. In addition to isotype switching and affinity maturation, helper T cells stimulate the production of long-lived plasma cells and the generation of memory B cells. Multivalent antigens with repeating determinants, such as polysaccharides, can activate B cells without T cell help. These antigens are called T-independent. T-independent responses are rapid but relatively simple, consisting mostly of low-affinity IgM antibodies, whereas T-dependent responses are slower to develop but result in more durable, high-affinity antibodies that are typically of the IgG, IgA, or IgE isotypes.

Primary and secondary antibody responses to protein antigens differ qualitatively and quantitatively (Fig. 12.3) **.** Primary responses result from the activation of previously unstimulated naive B cells, whereas secondary responses are due to the stimulation of expanded clones of memory B cells. Therefore, the secondary response develops more rapidly than does the primary response, and larger amounts of antibodies are produced in the secondary response. Furthermore, because the memory cells have already undergone isotype switching and some affinity maturation, there is more IgG and other isotypes compared to IgM and the affinity of the antibody is higher in secondary responses.

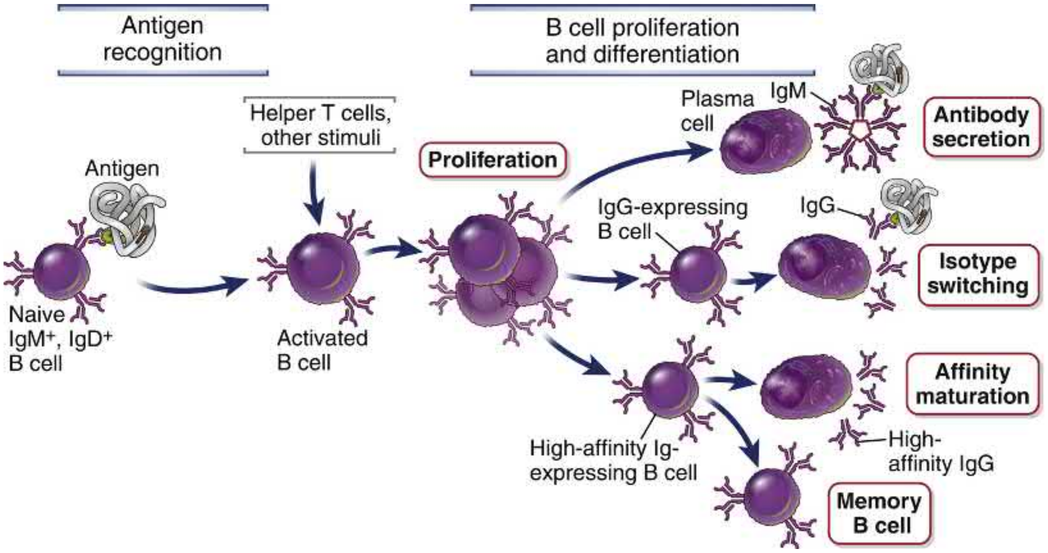

Antigen recognition
B cell proliferation and differentiation
Antigen
Naive IgM+, IgD+ B cell
Helper T cells, other stimuli
Activated B cell
Proliferation
Plasma cell
IgM
Antibody secretion
IgG-expressing B cell
IgG
Isotype switching
High-affinity Ig-expressing B cell
Affinity maturation
High-affinity IgG
Memory B cell

FIGURE 12.1 Phases of the humoral immune response. The activation of B cells is initiated by specific recognition of antigens by the surface immunoglobulin (Ig) receptors of the cells. Antigen and other stimuli, including helper T cells, stimulate the proliferation and differentiation of the specific B cell clone. Progeny of the clone may differentiate into plasma cells that produce IgM or other Ig isotypes (e.g., IgG), may undergo affinity maturation, or may persist as memory cells (that have also typically undergone class switching and affinity maturation).

Distinct subsets of B cells respond preferentially to different types of antigens (see Fig. 12.2) . Follicular B cells in secondary (peripheral) lymphoid organs make mostly antibody responses to protein antigens, and these B cell responses require collaboration with helper T cells. Marginal zone B cells in the spleen (and other lymphoid tissues in humans) and B-1 cells in mucosal tissues and the peritoneum recognize multivalent antigens, such as blood-borne polysaccharides, and mount primarily T-independent antibody responses. These preferences are not absolute. Some marginal zone B cells participate in T-dependent responses, and some follicular B cells may make T-independent responses.

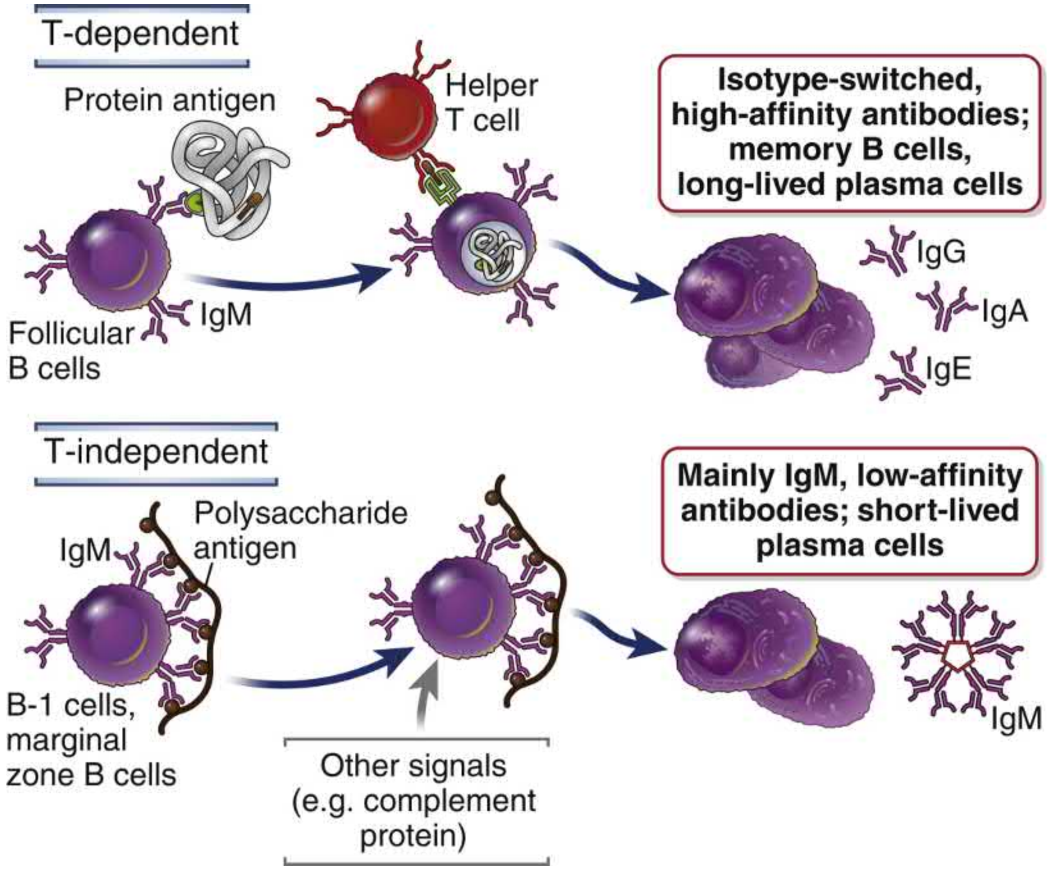
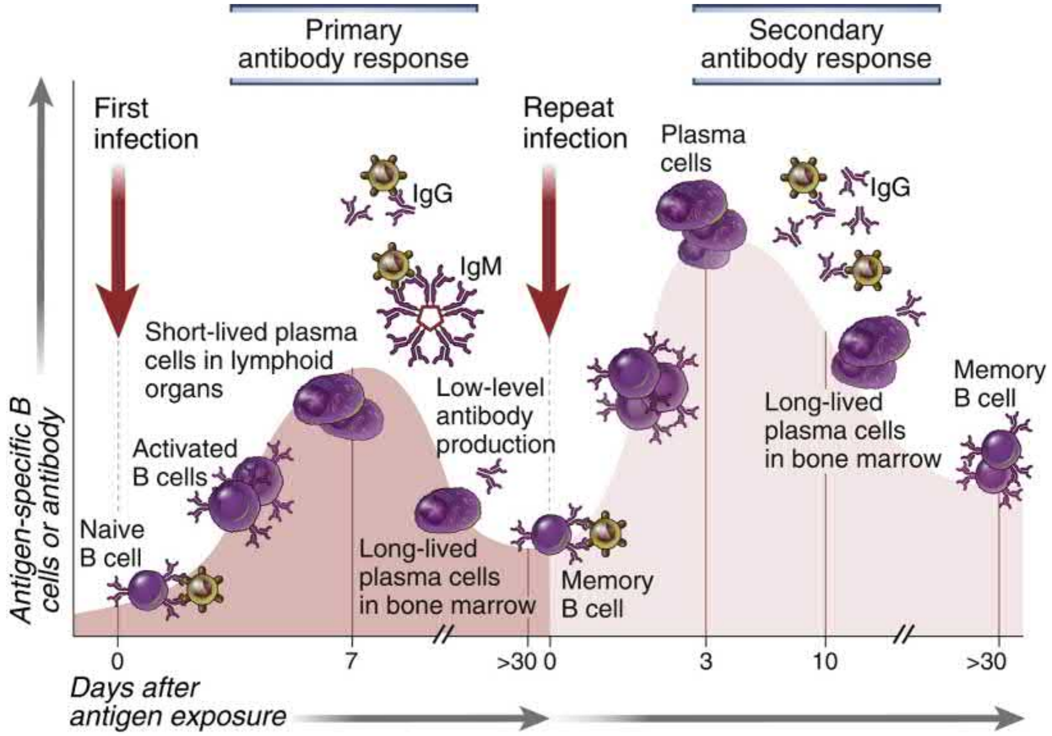

T-dependent
Protein antigen
Helper T cell
Isotype-switched, high-affinity antibodies; memory B cells, long-lived plasma cells
IgG
IgA
IgE
IgM
Follicular B cells
T-independent
Polysaccharide antigen
IgM
Mainly IgM, low-affinity antibodies; short-lived plasma cells
B-1 cells, marginal zone B cells
Other signals (e.g. complement protein)
IgM

FIGURE 12.2 T-dependent and T-independent antibody responses.T-dependent antibody responses to protein antigens mainly involve follicular B cells. T-independent responses to multivalent antigens are mediated mainly by marginal zone B cells in the spleen and B-1 cells in mucosal sites. *Ig,* Immunoglobulin.


Primary antibody response
Secondary antibody response
First infection
Repeat infection
Antigen-specific B cells or antibody
Naive B cell
Activated B cells
Short-lived plasma cells in lymphoid organs
IgG
IgM
Low-level antibody production
Long-lived plasma cells in bone marrow
Memory B cell
Plasma cells
IgG
Long-lived plasma cells in bone marrow
Memory B cell
0
7
>30
0
3
10
>30
Days after antigen exposure

Feature	Primary response	Secondary response
Magnitude	Smaller	Larger
Antibody isotype	Usually IgM > IgG	Relative increase in IgG, often IgA, and sometimes IgE
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)
Induced by	All antigens	Only protein antigens

FIGURE 12.3 Primary and secondary humoral immune responses. In a primary immune response, naive B cells are stimulated by antigen, become activated, and differentiate into antibody-secreting cells that produce antibodies specific for the eliciting antigen. A secondary immune response is elicited when the same antigen stimulates memory B cells, leading to production of greater quantities of specific antibody than are produced in the primary response. Note that the characteristics of secondary antibody responses summarized in the table are typical of T-dependent antibody responses to protein antigens. *Ig,* Immunoglobulin.

With this background, we proceed to a discussion of B cell activation, starting with the interaction of antigen with B cells. We will then describe the role of helper T cells in B cell responses to protein antigens and the mechanisms of isotype switching and affinity maturation. We conclude with a discussion of T-independent antibody responses.

Antigen Recognition and Antigen-Induced B Cell Activation

To initiate antibody responses, antigens have to be captured and transported to the B cell areas of secondary lymphoid organs. The antigens then initiate the process of B cell activation, often working in concert with other signals that are generated during innate immune responses triggered by microbes or by adjuvants in vaccines. We will next describe these early events in B cell activation.

Antigen Capture and Delivery to B Cells

Antigen may be delivered to naive B cells in lymphoid organs by multiple routes (Fig. 12.4) **.** Antigens that elicit antibody responses may vary in size and composition (they may be small, soluble, large, or particulate) and may be free or bound to antibodies. The major pathways of antigen delivery for different types of antigens include the following.

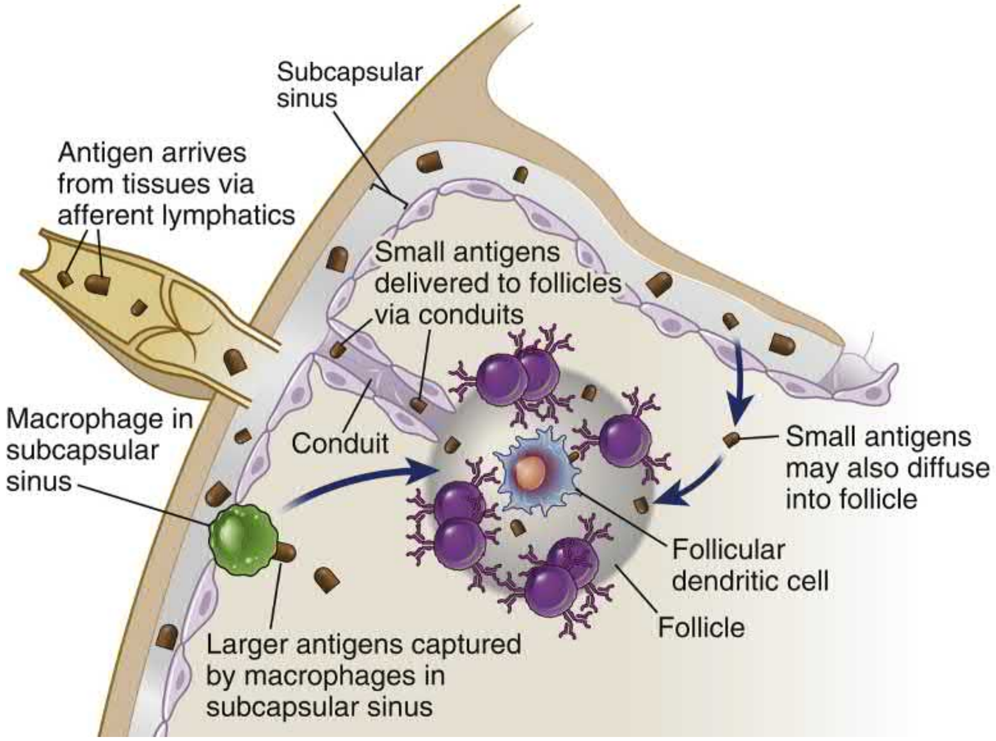

Subcapsular sinus
Antigen arrives from tissues via afferent lymphatics
Small antigens delivered to follicles via conduits
Macrophage in subcapsular sinus
Conduit
Small antigens may also diffuse into follicle
Follicular dendritic cell
Follicle
Larger antigens captured by macrophages in subcapsular sinus

FIGURE 12.4 Pathways of antigen delivery to follicular B cells.Small antigens are delivered to B cells in follicles through afferent lymphatics and via conduits and larger antigens by subcapsular sinus macrophages or by dendritic cells in the medulla.

- Most antigens from tissue sites are transported to lymph nodes by afferent lymphatic vessels that drain into the subcapsular sinus of the nodes. Soluble antigens, generally smaller than 70 kD, may then reach the B cell zone through conduits that extend between the subcapsular sinus and the underlying follicles (see Chapter 2). In some cases, small antigens may gain access to the follicles by diffusion.
- Subcapsular sinus macrophages capture large microbes and antigen-antibody complexes and deliver these to follicles.
- Antigens in immune complexes entering the spleen may bind to complement receptors (in particular, the complement receptor type 2 [CR2, CD21]) on marginal zone B cells, and these cells can transfer the immune complex–

containing antigens to follicular B cells. Recirculating follicular B cells may also capture antigens via CR2 and deliver them into follicles. Immune complexes that are delivered into lymphoid follicles may bind to CR2 or Fc receptors for IgG on the surface of follicular dendritic cells (FDCs), and the antigens in these complexes are then presented to antigen-specific B cells in a sustained manner (over days to weeks).
- Polysaccharide antigens can be captured by macrophages in the marginal zone of splenic lymphoid follicles and displayed or transferred to B cells in this area.

In all of these cases, ***the antigen that is presented to B cells is generally in its intact, native conformation and is not processed by antigen-presenting cells.*** This, of course, is one of the important differences between the forms of antigens recognized by B and T lymphocytes (see Chapter 6). Although the presentation of antigen to B cells by subcapsular sinus macrophages, macrophages in the splenic marginal zone, and dendritic cells (DCs) in the medulla of lymph nodes has been described in experimental models, how these cells prevent the protein antigens they capture from being engulfed and degraded remains unclear.

Activation of B Cells by Antigens and Other Signals

The B cell receptor (BCR) complex of mature B cells is composed of membrane Ig molecules that bind antigens and associated Igα and Igβ proteins that deliver signals for B cell activation. The BCR complex plays two key roles in B cell responses. First, binding of antigen to the receptor delivers biochemical signals to the B cells that initiate the process of activation. As discussed later, signaling is more robust with multivalent T-independent antigens than with T-dependent protein antigens. Antigen-induced biochemical signals are initiated by SRC family kinase–mediated phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) tyrosines of Igα and Igβ, followed by the recruitment and activation of SYK (see Chapter 7). Second, the BCR internalizes the bound antigen into endosomal vesicles, and if the antigen is a protein, it is processed into peptides that may be presented by class II MHC molecules on the B cell surface for recognition by helper T cells. This antigen-presenting function of B cells will be considered later in the context of T-dependent B cell activation.

Although antigen recognition can initiate B cell responses, by itself it is usually inadequate to stimulate significant B cell proliferation and differentiation, even for T-independent antigens. For full responses to be induced, other stimuli cooperate with BCR engagement, including complement proteins, pattern recognition receptors, and, in the case of protein antigens, helper T cells (discussed later).

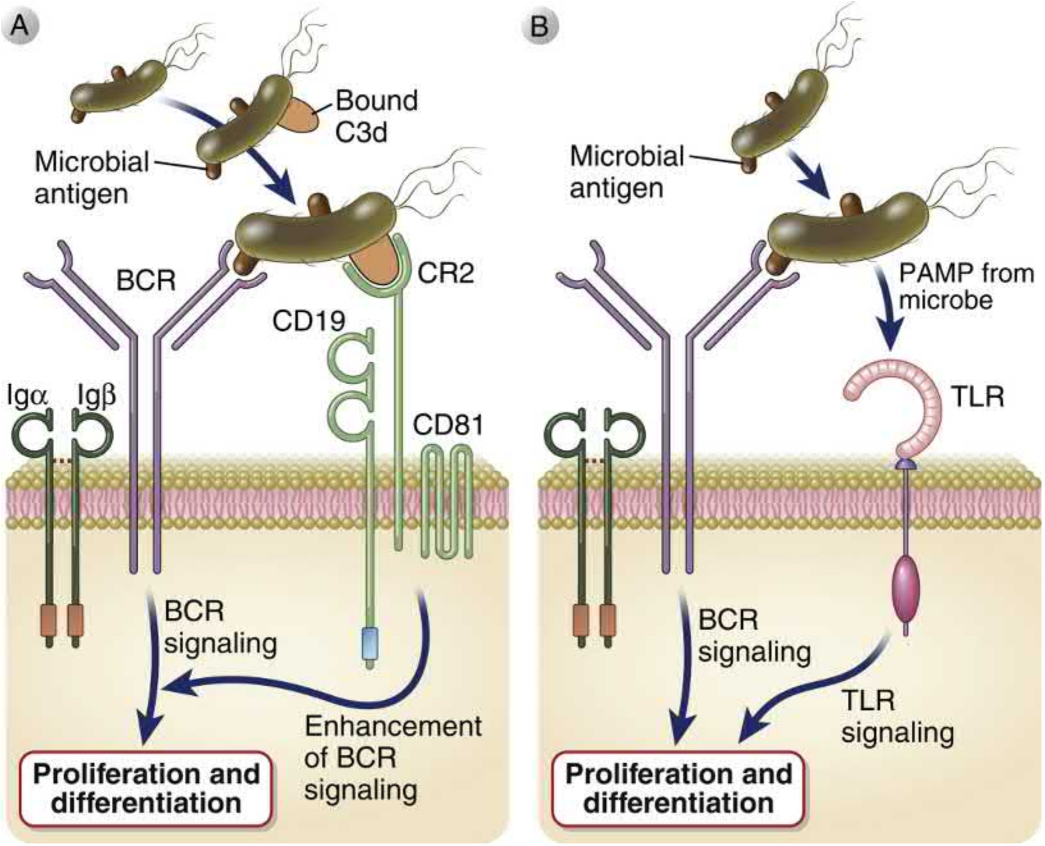

A
Microbial antigen
Bound C3d
BCR
CR2
CD19
Igα
Igβ
CD81
BCR signaling
Enhancement of BCR signaling
Proliferation and differentiation
B
Microbial antigen
PAMP from microbe
TLR
BCR signaling
TLR signaling
Proliferation and differentiation

FIGURE 12.5 Role of complement receptor type 2 and Toll-like receptors in B cell activation.In immune responses to microbes, activation of B cells through the B cell receptor *(BCR)* may be enhanced by complement-coated antigen that can ligate both the BCR and complement receptor 2 *(CR2)* **(A),** and also by the simultaneous activation of Toll-like receptors *(TLRs)* on B cells by molecules (pathogen-associated molecular patterns *[PAMPs]*) derived from the microbe **(B).**

B cell activation is facilitated by the CR2 coreceptor on B cells, which recognizes complement fragments that are covalently attached to the antigen or are part of immune complexes containing the antigen (Fig. 12.5A) **.** Follicular B cells and marginal zone B cells express the complement receptor CR2; the levels of CR2 on marginal zone B cells are much higher. Microbes activate the complement system in the absence of antibodies by the alternative and lectin pathways and in the presence of antibodies by the classical pathway (see Chapters 4 and 13). In all of these situations, complement fragments are generated that bind to the microbes. One of these fragments, called C3d, is recognized by CR2, which enhances the strength of BCR signaling and thus functions as a coreceptor for B cells (see Chapter 7). Some nonmicrobial polysaccharides also activate complement by the alternative or lectin pathway, and this is one reason that such antigens are able to induce antibody responses without T cell help.

Microbial products engage Toll-like receptors (TLRs) on B cells, which also enhances B cell activation (Fig. 12.5B) **.** Human B cells express several TLRs, including TLR5, which recognizes bacterial flagellin; endosomal TLR7, which recognizes single-stranded RNA; and TLR9, which is specific for unmethylated CpG-rich DNA in endosomes (see Chapter 4). Murine B cells (but not human B cells) also express TLR4 on the cell surface, which recognizes lipopolysaccharide (LPS). These pattern recognition receptors provide signals that enhance or cooperate with those from the BCR complex during B cell activation. In addition, the activation of myeloid cells through pattern recognition receptors can promote B cell activation indirectly in two ways. DCs activated through TLRs contribute significantly to helper T cell activation, and the helper cells stimulate B cells in response to protein antigens. Myeloid cells activated by TLRs may secrete APRIL (a proliferation-inducing ligand) and BAFF (B cell–activating factor), cytokines that can promote T-independent B cell responses.

The interaction of different types of antigens (multivalent structures or proteins) with the BCR initiates B cell proliferation and differentiation in different ways. Signals from the BCR may be sufficient to keep the B cells alive, induce changes in chemokine receptor expression, and promote antigen endocytosis (Table 12.1). The importance of signaling by the BCR complex for the subsequent responses of the cells varies with the nature of the antigen. Most T-independent antigens, such as polysaccharides, contain multiple identical epitopes on each molecule. Such multivalent antigens can effectively cross-link many B cell antigen receptors and initiate responses even though they are not recognized by helper T lymphocytes. In contrast, many naturally occurring globular protein antigens possess only one copy of each epitope per molecule. Therefore, such protein antigens, in their functionally monovalent form, cannot simultaneously bind to and cross-link multiple Ig molecules, and their ability to directly activate B cells is limited; they do not typically induce signals that can drive B cell proliferation and differentiation. Some protein antigens may be displayed as multivalent arrays on the surfaces of microbes or cells, or they may be multivalent because they are in aggregates; in these cases, protein antigens can induce B cell proliferation and differentiation.

TABLE 12.1

Effects of B Cell Antigen Receptor Engagement on B Cells [a]

Phenotypic Change	Functional Consequence
Increased expression of CCR7	Migration toward T cell zone
Increased expression of B7 costimulators	Enhanced ability to activate helper T cells
Increased expression of receptors for T-cell cytokines	Increased responsiveness to signals from helper T cells
Increased expression of anti-apoptotic proteins	Increased survival of B cells

[a] These changes may be induced by binding of protein antigens to the B cell receptor, and they prepare B cells to respond to T cell help. Protein antigens are also internalized, processed, and presented to helper T cells.

After specific B cells recognize antigens, the subsequent steps in humoral immune responses are very different in T-dependent and T-independent responses. We will next describe the activation of B cells by protein antigens and helper T cells.

Helper T Cell–Dependent Antibody Responses to Protein Antigens

The helper function of T lymphocytes was discovered by experiments performed in the late 1960s, which showed that antibody responses required cooperation between B cells and T cells. These classic experimental studies were among the first to demonstrate the importance of interactions between two different cell populations in the immune system. It was later established that most helper T cells are $CD4^{+}CD8^{-}$ lymphocytes that recognize peptide antigens presented by class II major histocompatibility complex (MHC) molecules. One of the important accomplishments of immunology has been the elucidation of the mechanisms of T cell–B cell interactions and the actions of helper T cells in antibody responses.

The Sequence of Events During T Cell–Dependent Antibody Responses

Protein antigens are independently recognized by specific B and T lymphocytes in secondary lymphoid organs, and the two activated cell types interact with each other to initiate humoral immune responses (Fig. 12.6) **.** Naive $CD4^{+}$ T cells are activated in the T cell zones by antigen (in the form of processed, MHC-associated peptides) presented by DCs. Naive B cells in the follicles are activated by the same antigen (in its native conformation) that is transported there. The activated helper T cells and activated B cells migrate toward one another and interact at the edges of the follicles, where the initial antibody response develops. Some of the activated T and B cells migrate into follicles to form germinal centers, where more specialized antibody responses are induced. Next we will describe each of these steps in detail.

Initial Activation and Migration of Helper B Cells and T Cells

The contemporaneous activation of specific B and T cells by a protein antigen induces changes that bring them into proximity to enhance the likelihood of the antigen-specific B and T cells colocalizing and interacting with one another (Fig. 12.7) **.** The frequency of naive B cells or T cells specific for a given epitope of an antigen is as low as 1 in 10^5 to 1 in 10^6 lymphocytes, and the specific B and T cells have to find each other and physically interact to generate strong antibody responses. This is accomplished in part by regulated movement of the cells following antigen recognition. Before antigen encounter, naive T cells reside in the T cell zone because they express the chemokine receptor CCR7, which binds to the chemokines CCL19 and CCL21 produced in these zones, and naive B cells remain in the follicles because they express CXCR5, which recognizes CXCL13 produced by FDCs and other cells in the follicles (see Chapter 2). Upon activation, helper T cells downregulate CCR7 and increase the expression of CXCR5 and, as a result, leave the T cell zone and migrate toward the follicle in response to CXCL13 in the follicle. B cells respond to antigen-mediated BCR triggering by reducing cell surface expression of the chemokine receptor CXCR5 and increasing expression of CCR7. These cells also upregulate EBI2, a chemoattractant receptor that responds to oxysterol ligands made along the follicle–T zone interface. (EBI2 is so named because it was discovered as an Epstein-Barr virus-induced gene.) Activated B cells thus migrate toward the T cell zone drawn by a gradient of CCL21, the major ligand for CCR7, and oxysterols. The net result of these changes is that antigen-activated T and B lymphocytes are drawn toward each other.

Protein antigens are internalized by the B cell and presented in a form that can be recognized by helper T cells, and this represents the next step in the process of T-dependent B cell activation.

Antigen Presentation by B Cells and the Hapten-Carrier Effect

Protein antigens that are recognized by specific BCRs are endocytosed and processed to generate peptides that bind to class II MHC molecules and are presented to CD4$^+$ T cells (Fig. 12.8) **.** This class II MHC pathway of antigen presentation was described in detail in Chapter 6. The peptides that are presented by the B cell to a helper T cell are the same peptides that initially activated the naive CD4$^+$ T cells when they were presented by DCs in the T cell zone. Because the BCR recognizes an epitope of the native protein with high affinity, specific B cells bind this antigen much more efficiently (i.e., at much lower concentrations) than do other B cells not specific for the antigen. Therefore, the antigen-specific B cells are also much more efficient at presenting peptides derived from that antigen than are other B cells that do not express membrane receptors for the antigen. This is why B cells specific for an antigen are best able to interact with helper T cells specific for that antigen and receive helper signals, whereas B cells with other BCRs remain in a quiescent state.

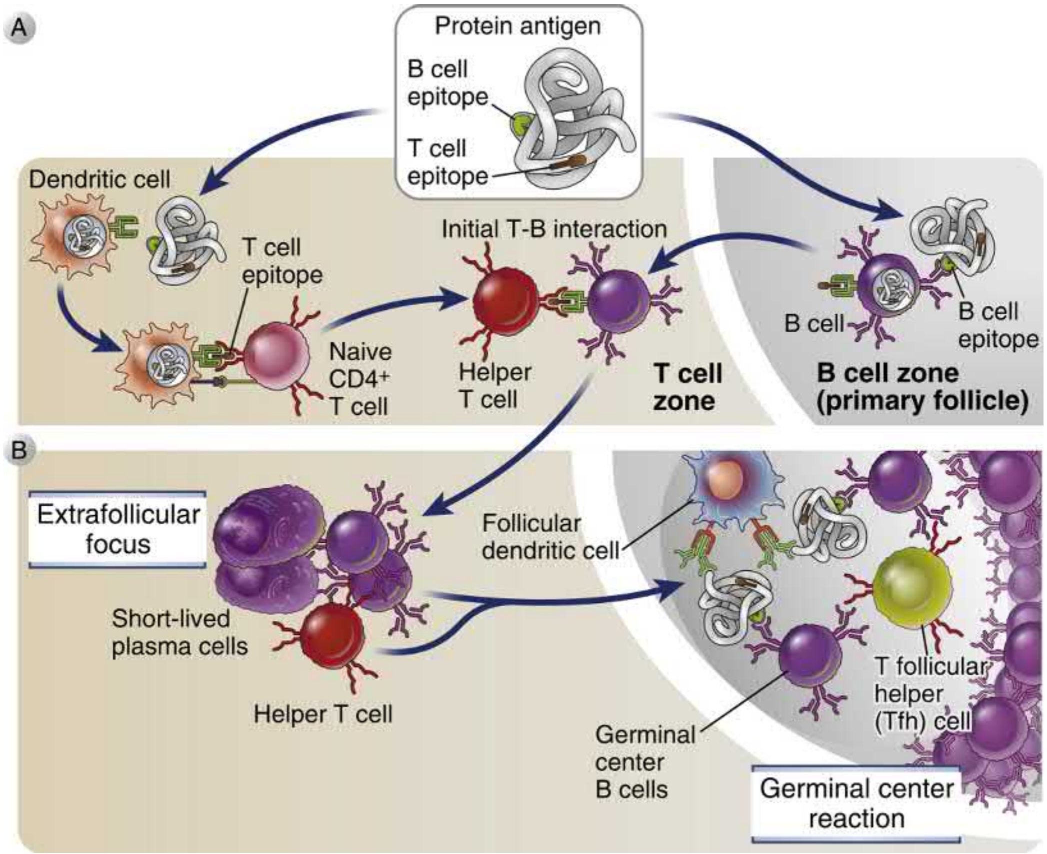

A
Protein antigen
B cell epitope
T cell epitope
Dendritic cell
T cell epitope
Initial T-B interaction
B cell
B cell epitope
Naive CD4+ T cell
Helper T cell
T cell zone
B cell zone (primary follicle)
B
Extrafollicular focus
Follicular dendritic cell
Short-lived plasma cells
T follicular helper (Tfh) cell
Helper T cell
Germinal center B cells
Germinal center reaction

FIGURE 12.6 Sequence of events in humoral immune responses to T cell–dependent protein antigens. **A,** Immune responses are initiated by the recognition of antigens by B cells and CD4$^+$ T cells. The activated lymphocytes migrate toward one another and interact at the interface of T and B cell zones. **B,** The initial T-dependent B cell proliferation and differentiation result in the formation of an extrafollicular focus, in which B cells proliferate, can undergo isotype switching, and differentiate into plasma cells (mostly short-lived). Some of the T cells that are activated in the extracellular focus develop into follicular helper T cells and migrate back into the follicles, together with some activated B cells, to form a germinal center. The late events in B cell responses occur in germinal centers and include somatic mutation

and the selection of high-affinity cells (affinity maturation), additional isotype switching, memory B cell generation, and the generation of long-lived plasma cells, described in later figures.

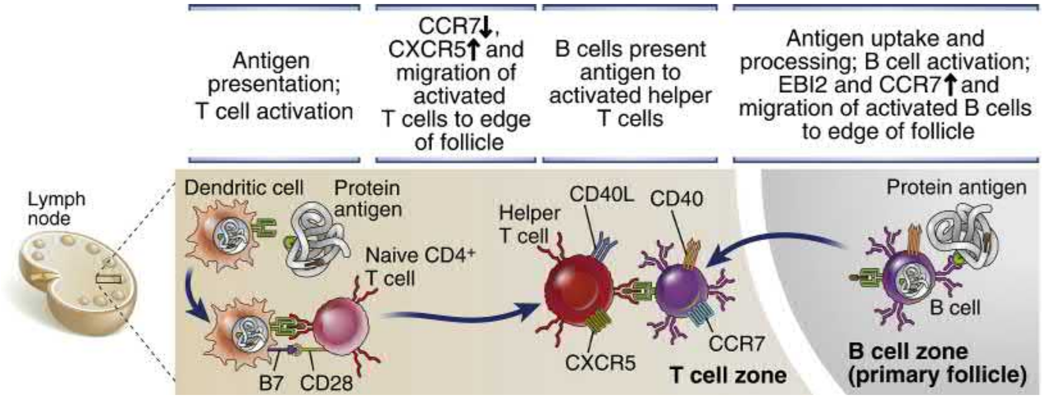

Antigen presentation; T cell activation
CCR7↓, CXCR5↑ and migration of activated T cells to edge of follicle
B cells present antigen to activated helper T cells
Antigen uptake and processing; B cell activation; EBI2 and CCR7↑ and migration of activated B cells to edge of follicle
Lymph node
Dendritic cell
Protein antigen
Naive CD4+ T cell
B7
CD28
Helper T cell
CD40L
CD40
CXCR5
CCR7
T cell zone
Protein antigen
B cell
B cell zone (primary follicle)

FIGURE 12.7 Migration of B cells and helper T cells and T cell–B cell interaction.Antigen-activated helper T cells and B cells move toward one another in response to chemokine signals and make contact adjacent to the edge of primary follicles. *CD40L,* CD40 ligand.

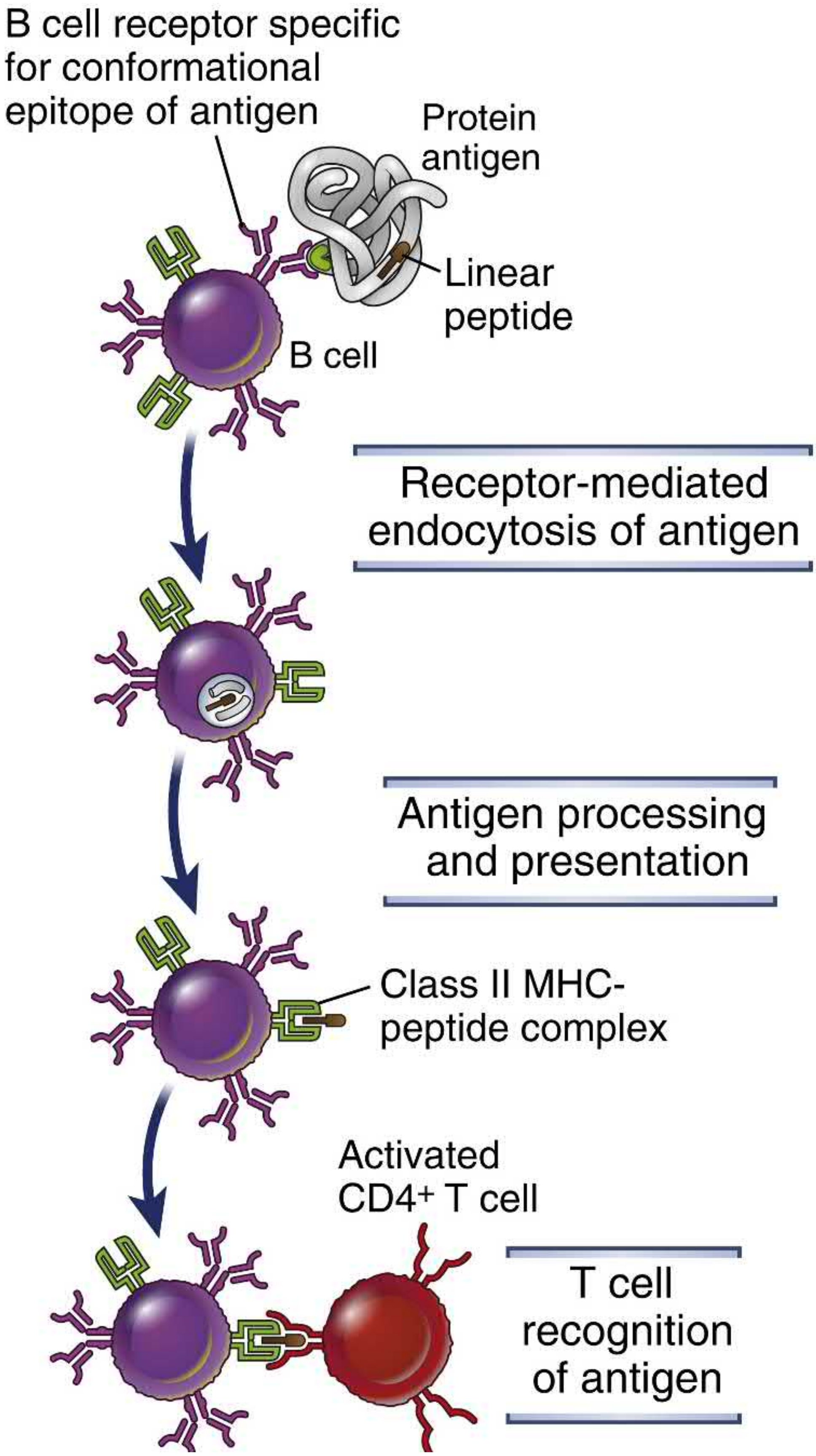

B cell receptor specific for conformational epitope of antigen
Protein antigen
Linear peptide
B cell
Receptor-mediated endocytosis of antigen
Antigen processing and presentation
Class II MHC-peptide complex
Activated CD4+ T cell
T cell recognition of antigen

FIGURE 12.8 Antigen presentation on B cells to helper T cells.Protein antigens recognized by membrane immunoglobulin are endocytosed and processed, and peptide fragments are presented in association with class II major histocompatibility complex *(MHC)* molecules. Helper T cells recognize MHC-peptide complexes on the B cells and then stimulate B cell responses.

In a T cell–dependent B cell response to a specific protein antigen, at least two different epitopes of the protein participate in the process: a surface epitope on the native protein is recognized with high specificity by a B cell; and a linear peptide epitope, which may be in any part of the intact protein, is subsequently released by proteolysis, binds to class II MHC molecules, and is recognized by helper T cells (see Fig 12.8). The antibodies that are eventually secreted are usually specific for conformational determinants of the native antigen, because membrane Ig on B cells is capable of binding conformational epitopes of proteins, and the same Ig is secreted by plasma cells derived from those B cells. This feature of B cell antigen recognition determines the fine specificity of the antibody response and is independent of the fact that helper T cells recognize only linear epitopes of processed peptides. In fact, a single B lymphocyte specific for a native epitope may bind and endocytose a protein and present multiple different peptides complexed with class II MHC molecules to different helper T cells, but the resultant antibody response remains specific for the native protein.

The principles outlined here for T cell–B cell collaboration help to explain a phenomenon that is known as the **hapten-carrier effect.** Haptens, such as dinitrophenol, are small chemicals that can be recognized by specific antibodies but are not immunogenic by themselves. If, however, haptens are coupled to proteins, which serve as carriers, the conjugates are able to induce antibody responses against the haptens. The haptens are equivalent to the conformational epitopes that are recognized by B cells in a T-dependent response to any protein antigen. Analysis of antibody responses to hapten-carrier conjugates provided among the earliest demonstrations of how antigen presentation by B lymphocytes contributes to the development of humoral immune responses. There are three important characteristics of anti-hapten antibody responses to hapten-protein conjugates. First, such responses require both hapten-specific B cells and protein (carrier)-specific helper T cells. Second, to stimulate a response, the hapten and carrier portions have to be physically linked and cannot be administered separately. Third, the interaction is class II MHC restricted; that is, the helper T cells cooperate only with B lymphocytes that express class II MHC molecules that are identical to those that were involved in the initial activation of naive T cells by DCs. All of these features of antibody responses to hapten-protein conjugates can be explained by the antigen-presenting functions of B lymphocytes. Hapten-specific B cells bind the antigen through the hapten determinant, endocytose the hapten-carrier conjugate, digest the protein component, and present peptides derived from the carrier protein to carrier-specific helper T lymphocytes (see Fig. 12.8). Thus, the two cooperating lymphocytes recognize different epitopes of the same antigen. The hapten is responsible for efficient internalization of the carrier protein into the B cell, which explains why hapten and carrier must be physically linked. Because the T cells recognize self MHC-associated peptides, their interaction with the B cells is MHC-restricted.

The characteristics of humoral responses elucidated for hapten-carrier conjugates apply to all protein antigens in which one intrinsic determinant, usually a native conformational determinant, is recognized by B cells (and is therefore analogous to the hapten), and another determinant, in the form of a class II MHC–associated linear peptide, is recognized by helper T cells (and is analogous to a peptide derived from the carrier protein). The hapten-carrier effect is the basis for the development of conjugate vaccines against encapsulated bacteria; these vaccines contain carbohydrate epitopes recognized by B cells attached to proteins recognized by T cells, discussed later in this chapter.

Role of CD40L:CD40 Interaction in T-Dependent B Cell Activation

Upon activation, helper T cells express CD40 ligand (CD40L), which engages its receptor, CD40, on antigen-stimulated B cells and induces B cell proliferation and differentiation, initially in extrafollicular foci and later in germinal centers (Fig. 12.9) **.** CD40 is a member of the tumor necrosis factor (TNF) receptor superfamily (see Chapter 10). Its ligand, CD40L (CD154), is a trimeric membrane protein that is homologous to TNF. CD40 is constitutively expressed on B cells, and CD40L is expressed on the surface of helper T cells that have been recently activated by antigen and costimulators. When these activated helper T cells interact physically with antigen-presenting B cells, CD40L binds to CD40 on the B cell surface. This results in conformational alteration of preformed CD40 trimers, which induces the association of cytosolic proteins called TNF receptor–associated factors (TRAFs) with the cytoplasmic domain of CD40. The TRAFs recruited to CD40 initiate enzyme cascades that lead to the activation and nuclear translocation of transcription factors, including NF-κB (nuclear factor κB) and AP1 (activator protein 1), which collectively stimulate B cell proliferation and increased synthesis and secretion of Ig. Similar signaling pathways are activated by TNF receptors (see Chapter 7). CD40-induced signals are also crucial for subsequent germinal center reactions, as we will discuss later. In addition, T cell–mediated DC and macrophage activation involves the interaction of CD40L on activated helper T cells with CD40 on DCs and macrophages (see Chapters 6 and 10).

Mutations in the *CD40L* gene result in a disease called the **X-linked hyper-IgM syndrome,** which is characterized by defects in antibody production, notably in isotype switching and affinity maturation, and deficient cell-mediated immunity (see Chapter 21).

Similar abnormalities are seen in *CD40* or *CD40L* gene knockout mice. Interestingly, a DNA virus called the Epstein-Barr virus (EBV) infects human B cells and induces their proliferation. This may lead to immortalization of the cells and the development of lymphomas. The cytoplasmic tail of the EBV protein latent membrane protein 1 (LMP1) associates with the same TRAF molecules as does the cytoplasmic domain of CD40, and this apparently triggers B cell proliferation. Thus, EBV LMP1 is functionally homologous to a physiologic B cell signaling molecule, and EBV has apparently co-opted a normal pathway of B lymphocyte activation for its own purpose, which is to promote survival and proliferation of cells in which the virus can replicate.

In addition to CD40L on helper T cells activating B cells, helper T cells also secrete cytokines that contribute to B cell responses. T cell–derived cytokines are essential for germinal center reactions, described later. Several cytokines also have been implicated in the early steps of B cell proliferation and differentiation, but it is not clear if any are actually essential for these responses.

After the initial interaction of B cells with helper T cells at the interface between the follicle and the T cell zone, subsequent activation of B cells by helper T cells can occur at two different locations, one outside the follicles in an extrafollicular focus and the other in the germinal centers of follicles. The nature of the B cell response differs in these locations (Table 12.2).

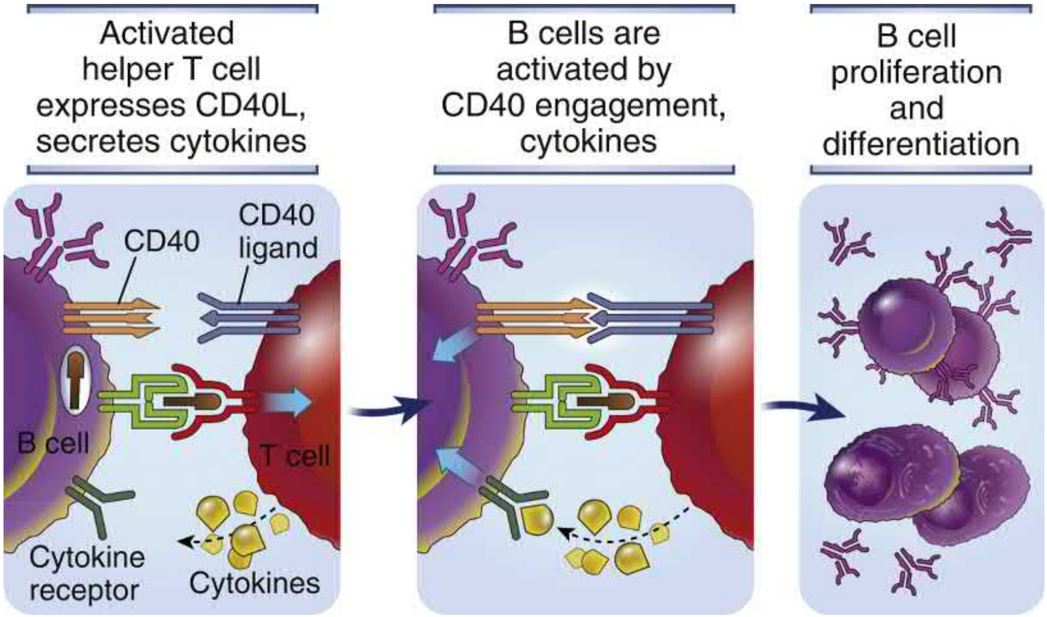

Activated helper T cell expresses CD40L, secretes cytokines
B cells are activated by CD40 engagement, cytokines
B cell proliferation and differentiation
CD40
CD40 ligand
B cell
T cell
Cytokine receptor
Cytokines

FIGURE 12.9 Mechanisms of helper T cell–mediated B cell activation.Helper T cells that are activated by recognizing antigens presented by B cells express CD40 ligand *(CD40L)*, which binds to CD40 on B cells and stimulates B cell proliferation and differentiation. Cytokines produced by the helper T cells also contribute to B cell responses.

Extrafollicular B Cell Activation

B cell activation in the extrafollicular focus results in an early antibody response to protein antigens and sets up the subsequent germinal center reaction. Extrafollicular foci of T-dependent B cell activation generate low-affinity antibodies that can circulate and limit the spread of an infection. Each such focus may produce 100 to 200 antibody-secreting plasma cells. In the spleen, extrafollicular foci develop in the outer portions of the T cell–rich periarteriolar lymphoid sheath (PALS) or between the T cell zone and the red pulp, and these collections of cells are also called PALS foci. Similar T-dependent foci are observed in the medullary cords of lymph nodes.

B cells that are activated by helper T cells through CD40L in the extrafollicular foci undergo isotype switching. In fact, extracellular foci are the site at which most isotype switching occurs during antibody responses to protein antigens. The antibody-secreting cells that are generated in extrafollicular foci, including plasmablasts and tissue plasma cells, are mostly short-lived, and these cells do not acquire the ability to migrate to distant sites, such as the bone marrow. In secondary lymphoid organs, plasmablasts downregulate CXCR5 and CCR7, upregulate CXCR4, and migrate into the red pulp (in the spleen) or medullary cords (in lymph nodes) in response to the CXCR4 ligand CXCL12. Rather than taking on plasmablast properties, some of the B cells activated at the follicle–T zone interface become precursors of germinal center B cells. FDCs release CXCL13, which draws in small numbers (a few to 100) of such cells. These precursor cells downregulate EBI2 and upregulate receptors that promote their confinement to the follicle center and initiate the germinal center reaction. The extrafollicular response also helps to generate **T follicular helper (Tfh) cells** that migrate into the follicle and are required for germinal center formation.

T Follicular Helper (Tfh) Cells

Within 4 to 7 days after antigen exposure, activated antigen-specific B cells outside the follicle induce some previously activated T cells to differentiate into Tfh cells, which express high levels of the chemokine receptor CXCR5, are drawn into lymphoid follicles by CXCL13, the ligand for CXCR5, and play critical roles in germinal center formation and function. In addition to CXCR5, Tfh cells express ICOS (inducible costimulator), PD-1 (programmed cell death protein-1), the cytokine interleukin-21 (IL-21), and the transcription factor BCL-6. Tfh cells have a phenotype that makes them distinct from the Th1, Th2, and Th17 subsets of effector T cells described in Chapter 10.

Differentiation of Tfh cells from naive CD4$^+$ T cells requires two steps: initial activation by antigen-presenting DCs and subsequent activation by B cells (Fig. 12.10). The choice between a Th1, Th2, or Th17 fate on the one hand or a Tfh fate on the other depends partly on the strength of the initial interaction between peptide–class II MHC complexes on DCs and the T cell receptor (TCR) on naive CD4$^+$ T cells. Strong TCR activation by DCs induces Tfh cells by promoting expression of the BCL-6 transcriptional repressor and reducing the levels of the α chain of the IL-2 receptor (IL-2R). This initial expression of BCL-6 combined with weak IL-2R signaling inhibits the acquisition of a Th1, Th2, or Th17 cell fate. Some of these activated T cells begin to express CXCR5, and their final differentiation into Tfh cells requires interacting with activated B cells. A number of molecules on B cells and helper T cells are known to play key roles in the generation of Tfh cells. The binding of the costimulatory molecule ICOS ligand on activated B cells to its receptor ICOS, a member of the CD28 family, on T cells promotes the differentiation of the T cells into Tfh cells. Tfh cell generation also depends on interactions between activated B cells and helper T cells mediated by members of the SLAM family. A signaling molecule that associates with these SLAM family proteins in Tfh cells is called SLAM-associated protein (SAP), and SAP signaling stabilizes the expression of transcriptional regulators, particularly BCL-6, that are required for Tfh cell development. SAP is mutated in patients with **X-linked lymphoproliferative syndrome (XLP),** which is associated with defects in antibody and cytotoxic T cell responses (see Chapter 21).

TABLE 12.2

Extrafollicular and Germinal Center B Cell Responses

Feature	Extrafollicular Response	Germinal Center Response
Localization	Medullary cords of lymph nodes and at junctions between T cell zone and red pulp of spleen	Germinal centers of secondary follicles
CD40 signals	Required	Required
Specialized T cell help	Extrafollicular helper T cells	Tfh cells in germinal center
AID expression	Yes	Yes
Isotype switching	Yes	Yes
Somatic hypermutation	Low rate	High rate
Affinity maturation of antibody	Low	High
Terminally differentiated B cells	Short-lived plasma cells (life span of ∼3 days)	Long-lived plasma cells, which migrate to bone marrow, and memory cells
Transcription factors activated in B cells	BLIMP-1	BCL-6

AID, Activation-induced cytidine deaminase; *BCL-6*, B cell lymphoma 6; *BLIMP-1*, B lymphocyte–induced maturation protein 1; *Tfh*, T follicular helper cell.

Data from Vinuesa CG, Sanz I, Cook MC. Dysregulation of germinal centres in autoimmune disease. *Nat Rev Immunol*. 2009;9:845–885.

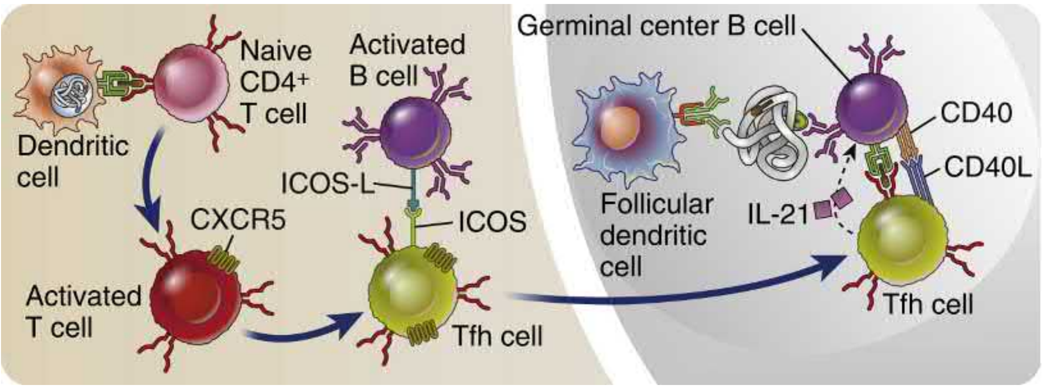

Dendritic cell
Naive CD4+ T cell
Activated B cell
Germinal center B cell
CD40
CD40L
ICOS-L
ICOS
CXCR5
Follicular dendritic cell
IL-21
Activated T cell
Tfh cell
Tfh cell

FIGURE 12.10 The generation of T follicular helper cells. The generation of T follicular helper *(Tfh)* cells requires sequential activation of T cells, first by dendritic cells and then by activated B cells. Inducible costimulator (ICOS) ligand *(ICOS-L)*-ICOS interactions are essential for Tfh cell differentiation. The differentiated Tfh cells migrate into germinal centers, where they activate B cells. *IL*, Interleukin.

The defining cytokine produced by Tfh cells is IL-21. This cytokine is required for germinal center development and contributes to the generation of plasma cells in the germinal center reaction. IL-21 secreted by Tfh cells also facilitates germinal center B cell selection events and the differentiation of activated B cells into plasmablasts. In addition to IL-21, Tfh cells secrete other cytokines, including IL-4 and IL-13 and likely low levels of IFN-γ as well; some of these cytokines are known to participate in isotype switching, as discussed later. Some extrafollicular Tfh cells may express cytokines such as IL-4 and IL-21, which stimulate isotype switching outside the germinal center, as described shortly.

The Germinal Center Reaction

The characteristic events of helper T cell–dependent antibody responses, including affinity maturation, generation of long-lived plasma cells and memory B cells, and continuing isotype switching, occur in organized structures called germinal centers that are created within lymphoid follicles during T-dependent immune responses. The complex process of B cell differentiation and selection of cells with the highest affinity antigen receptors that occurs in these sites is called the germinal center reaction.

Germinal centers develop in secondary lymphoid organs approximately 4 to 7 days after the initiation of a T-dependent B cell response. Long before the functional significance of this anatomic structure was understood it was named the germinal center by morphologists because the presence of many mitotic figures in the region suggested that new cells were generated or "germinated" there. Germinal centers consist of two distinct regions: a **dark zone** that is densely packed with rapidly proliferating B cells and appears dark in histologic sections stained with hematoxylin; and a **light zone,** where high-affinity B cells are selected to survive and differentiate further but many cells die, that stains weakly with the same dye (Fig. 12.11). The cellular composition of these zones is different. Tfh cells are present only in the light zone. The light zone also contains a type of stromal cell called a **follicular dendritic cell (FDC).** FDCs are not derived from bone marrow precursors and are developmentally related to fibroblastic reticular cells. They are found only in lymphoid follicles and express complement receptors (CR1, CR2, and CR3) and Fc receptors. These molecules are involved in displaying antigens for the selection of germinal center B cells, as described later. FDCs do not express class II MHC molecules and, in spite of their name, they are distinct from the class II MHC–expressing DCs that present peptides to T lymphocytes. The long cytoplasmic processes of FDCs form a meshwork around which germinal centers are formed. B cells in the dark zone and light zone are sometimes called centroblasts and centrocytes, respectively. These zones are not fixed, because B cells move between the dark zone and the light zone. The rim of naive B cells in the follicle surrounding the germinal center makes up the mantle zone.

The germinal center reaction consists of a number of sequential steps (Fig. 12.12).

1. *Initiation of the germinal center by Tfh cells:* The germinal center reaction begins with the migration of Tfh cells into the follicle, guided by the chemokine CXCL13 blinding to CXCR5 on the T cells.
2. *Entry of B cells into the GC:* Antigen-stimulated B cells that have been activated in extrafollicular foci cells through CD40L-CD40 interactions downregulate EBI2 and move into the follicle to form the germinal center. Each germinal center contains B cells derived from as little as a few to up to about a hundred antigen-specific clones. Different antigen-specific B cell clones may give rise to memory B cells and long-lived plasma cells.
3. *B cell proliferation:* B cells that have been triggered by Tfh cells via CD40L-CD40 interactions repeatedly proliferate, forming the dark zone of the germinal center. The doubling time of these proliferating germinal center B cells is estimated to be 6 to 12 hours, so that within 5 days, a single lymphocyte may give rise to as many as 5000 progeny.
4. *Somatic mutations in Ig genes:* Proliferating dark zone B cells mutate their Ig V genes as part of a process called somatic hypermutation. This process is initiated by the enzyme activation-induced cytidine deaminase (AID), which is highly expressed by dark zone B cells. The mechanism of somatic mutation is described later.
5. *B cell migration within the GC:* After multiple divisions, dark zone B cells stop dividing and shut off expression of the CXCR4 chemokine receptor. The CXCR4 ligand, CXCL12, is

more abundant in the dark zone than in the light zone. The loss of CXCR4 expression results in the migration of these heavily mutated nondividing B cells to the adjacent light zone that contains Tfh cells and FDCs. These nondividing dark zone B cells still express CXCR5 and are drawn toward the higher concentration of CXCL13 in the light zone.

6. *Selection of high-affinity B cells:* In the light zone, B cells that have undergone somatic hypermutation are tested for their ability to recognize antigen displayed by FDCs and free antigen in the GC. B cells that have acquired high-affinity BCRs are more likely to capture antigen, display the antigen to Tfh cells, and receive CD40- and cytokine-mediated signals from the T cells. As a result, these B cells are selected to survive and differentiate further. B cells may fail selection and undergo apoptotic death in the light zone. This selection process is also described in more detail later.
7. *Repetitive mutation and selection:* B cells positively selected in the light zone express the transcription factor MYC, reexpress the CXCR4 chemokine receptor and thus return to the dark zone. Germinal center B cells can undergo repeated rounds of mutation and selection, migrating back and forth from light to dark zone if they are positively selected. This process contributes to the affinity maturation of the antibody response.
8. *Differentiation into long-lived plasma cells:* After some rounds of selection, high-affinity B cells exit the germinal center as plasmablasts that will home to the bone marrow and differentiate into long-lived plasma cells. How the decision is made for selected high-affinity light zone B cells to not return to the dark zone but to instead exit the germinal center is not known. What is known is that once this decision is made, high-affinity light zone B cells reexpress EBI2 and leave the germinal center.
9. *Memory B cell formation: From the germinal center,* B cells that undergo limited somatic hypermutation and remain relatively low-affinity exit early as memory B cells. Memory

cells have the ability to recirculate and migrate from one secondary lymphoid organ to another.

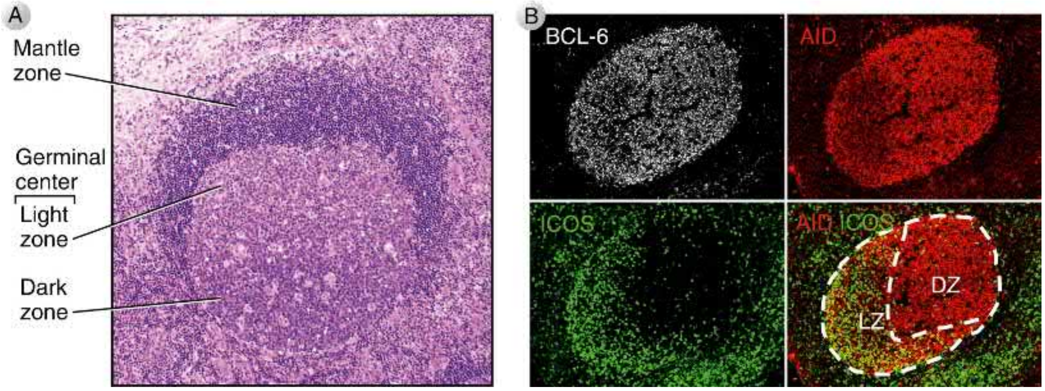


FIGURE 12.11 Germinal centers in secondary lymphoid organs. **A,** The germinal center is within the follicle and includes a basal dark zone *(DZ)* and an adjacent light zone *(LZ).* **B,** Germinal center B cells express *BCL-6* (B cell lymphoma 6) (as do T follicular helper cells); *ICOS* (inducible costimulator) expressing helper T cells reside mainly in the light zone and in the extrafollicular area; and *AID* (activation-induced cytidine deaminase) is expressed at its highest levels in germinal center B cells in the dark zone.

A, Courtesy of Dr. James Gulizia, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts. *B,* Courtesy of Dr. Naoki Kaneko, Ragon Institute of MGH, MIT and Harvard, Cambridge, Massachusetts.

Germinal center formation is defective in humans and in mice with genetic defects in T cell development or activation or with mutations of either CD40 or its ligand, discussed earlier. Now that we have described the basic features and steps of the germinal center reaction, we will discuss some of the individual cellular and molecular events that occur during this process.

Heavy Chain Isotype (Class) Switching

In T-dependent responses, some of the progeny of activated IgM- and IgD-expressing B cells undergo heavy chain isotype (class) switching and produce antibodies with heavy chains of different classes, such as γ, α, and ε (Fig. 12.13) **.** Isotype switching outside the follicle is driven by extrafollicular pre-germinal center Tfh cells, and the process continues to occur in germinal centers, driven by Tfh cells in the light zone. The capacity of B cells to produce different antibody isotypes provides a remarkable specialization of humoral immune responses by generating antibodies that perform distinct effector functions and are involved in defense against different types of infectious agents. B cells change the isotypes of the antibodies they produce by changing the constant regions of the heavy chains, but the specificity of the antibodies (which is determined by the variable regions) remains unaltered. The molecular mechanisms responsible for the change in heavy chain constant regions are described later.

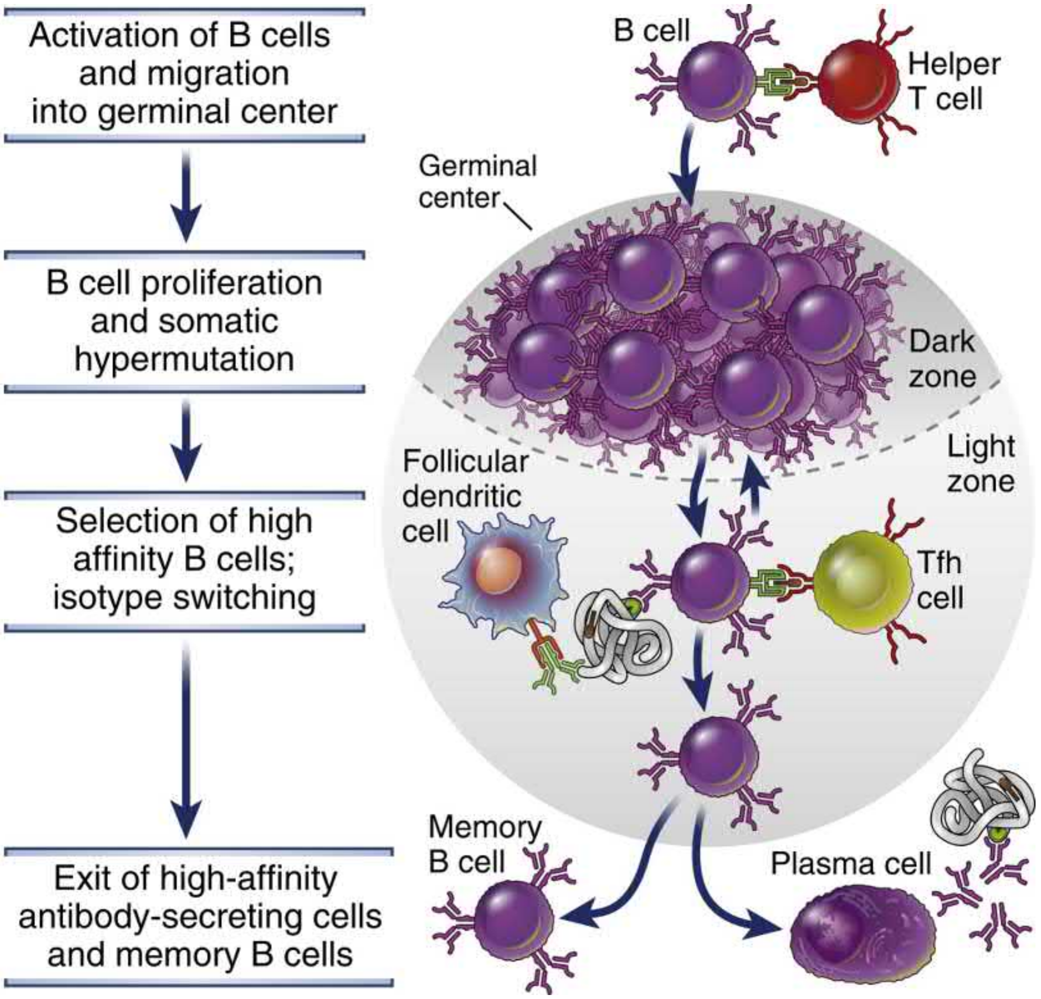

Activation of B cells and migration into germinal center
B cell proliferation and somatic hypermutation
Selection of high affinity B cells; isotype switching
Exit of high-affinity antibody-secreting cells and memory B cells
B cell
Helper T cell
Germinal center
Dark zone
Light zone
Follicular dendritic cell
Tfh cell
Memory B cell
Plasma cell

FIGURE 12.12 Sequential events in the germinal center.Activated B cells migrate into the follicle and proliferate, forming the dark zone of the germinal center. These B cells undergo somatic hypermutation of immunoglobulin *(Ig)* V genes and migrate into the light zone, where they encounter follicular dendritic cells displaying antigen and T follicular helper *(Tfh)* cells. B cells with the highest-affinity Ig receptors are selected to survive, and cycle back to the dark zone where they undergo more somatic hypermutation. Selected cells go back and forth multiple times and are repeatedly mutated and selected until they finally differentiate into antibody-secreting

cells. Memory B cells are derived from B cells that go through fewer rounds of somatic hypermutation and selection and emerge earlier from the germinal center than antibody-secreting cells. The antibody-secreting cells leave and reside in the bone marrow as long-lived plasma cells, and the memory B cells enter the recirculating lymphocyte pool.

Isotype switching in response to different types of pathogens is regulated by cytokines produced by the helper T cells that are activated by these pathogens.

- Switching from IgM to **IgG** is a prominent aspect of T-dependent antibody responses against many bacteria and viruses. IgG antibodies not only promote phagocytosis of opsonized microbes and activate complement but are also transferred through the placenta to protect newborns, and they have longer half-lives in the blood than other isotypes, so the production of IgG contributes in many ways to the protective capacity of humoral immunity. In mice, switching to IgG subclasses is induced by the cytokine IFN-γ, which may be produced by Tfh cells activated by these microbes. However, there is no evidence that in humans, isotype switching to IgG involves IFN-γ. Human Tfh cells that express higher levels of IL-21 relative to IL-4 drive the IgM to IgG class switch but the details of which cytokines drive switching differentially to IgG1, IgG2, IgG3, and IgG4 remain to be ascertained.
- The humoral response to many helminthic parasites is dominated by **IgE** antibodies, which participate in elimination of the helminths (see Chapters 13 and 16); IgE antibodies also mediate immediate hypersensitivity (allergic) reactions (see Chapter 20). Helminths likely influence Tfh cell differentiation and induce these helper T

cells to produce Th2-type cytokines IL-4 and IL-13, which drive isotype switching from IgM to IgE.

- In addition, B cells in different anatomic sites switch to different isotypes, in part because of the cytokines produced at these sites. Specifically, B cells in mucosal tissues switch to **IgA,** which is the antibody class that is most efficiently transported through epithelia into mucosal secretions, where it prevents microbes from entering through the epithelia (see Chapter 14). Switching to IgA is stimulated by transforming growth factor-β (TGF-β), which is produced by many cell types, including helper T cells, in mucosal and other tissues. Cytokines of the TNF family, BAFF and APRIL, also stimulate switching to IgA. Because these cytokines are produced by myeloid cells, they can stimulate IgA responses in the absence of T cell help. Some individuals who inherit mutant versions of the *TACI* gene, which encodes a receptor for these cytokines, have a selective deficiency of IgA production (see Chapter 21).

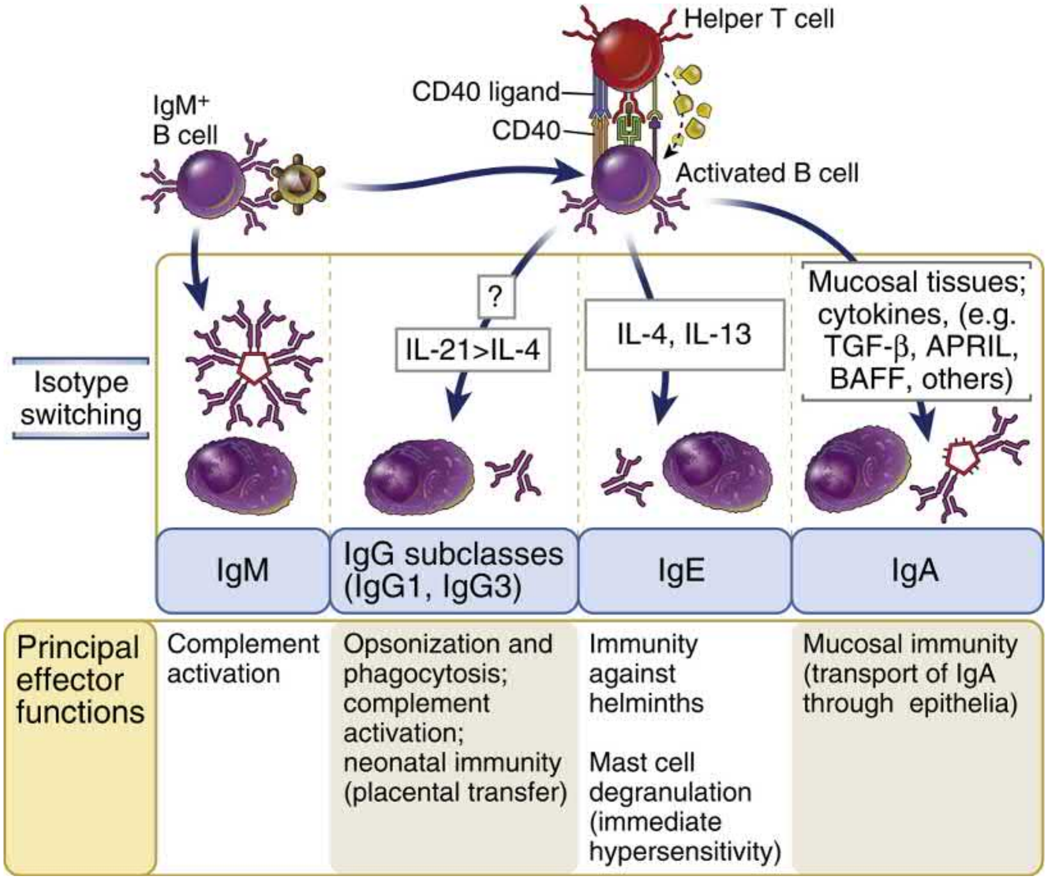

Helper T cell
CD40 ligand
CD40
IgM+ B cell
Activated B cell
Isotype switching
?
IL-21>IL-4
IL-4, IL-13
Mucosal tissues; cytokines, (e.g. TGF-β, APRIL, BAFF, others)
IgM
IgG subclasses (IgG1, IgG3)
IgE
IgA
Principal effector functions
Complement activation
Opsonization and phagocytosis; complement activation; neonatal immunity (placental transfer)
Immunity against helminths
Mast cell degranulation (immediate hypersensitivity)
Mucosal immunity (transport of IgA through epithelia)

FIGURE 12.13 Immunoglobulin heavy chain isotype switching.B cells activated by helper T cell signals (CD40, cytokines) undergo switching to different immunoglobulin isotypes, which mediate distinct effector functions. Selected examples of switched isotypes are shown. All isotypes are capable of neutralizing microbes and toxins. *APRIL,* A proliferation-inducing ligand; *BAFF,* B cell–activating factor; *Ig,* immunoglobulin; *IL,* interleukin; *TGF-β,* transforming growth factor-β.

CD40 signals work together with cytokines to induce isotype switching. CD40 engagement induces the expression of the enzyme AID, which, as we will see later, is crucial for both isotype switching and affinity maturation. The requirement for CD40 signaling and AID to promote isotype switching in B cells is well documented by analysis of mice and humans lacking CD40, CD40L, or AID. In all of these cases, the antibody response to protein antigens is dominated by IgM antibodies, and there is limited switching to other isotypes.

The molecular mechanism of isotype switching is a process called switch recombination, in which the Ig heavy chain DNA in B cells is cut and recombined such that a previously formed VDJ exon that encodes the V domain is placed adjacent to a downstream C region, and the intervening DNA is deleted (Fig. 12.14) **.** These DNA recombination events involve nucleotide sequences called switch regions, which are located in the introns between the J and C segments at the 5′ ends of each C_H locus, other than the δ gene. Switch regions are 1 to 10 kilobases long, contain numerous tandem repeats of GC-rich DNA sequences, and are found upstream of heavy chain genes. Upstream of each switch region is a small I exon (for initiator of transcription) preceded by an I region promoter. Signals from cytokines induce transcription from a particular I region promoter reading through the I exon, switch region, and adjacent C_H exons. These transcripts are known as germline transcripts because they are not translated into proteins, but they are required for isotype switching to proceed. Germline transcripts are found at both the μ locus and the downstream heavy chain locus to which an activated B cell is being induced to switch. At each participating switch region, the germline transcript facilitates the generation of DNA double-strand breaks, as described later. The DNA break in the upstream (μ) switch region is joined to the break in the downstream selected switch region. As a result, the rearranged VDJ exon just upstream of the μ switch region in the IgM-producing B cell recombines with the Ig heavy chain gene located immediately after the transcriptionally active downstream switch region.

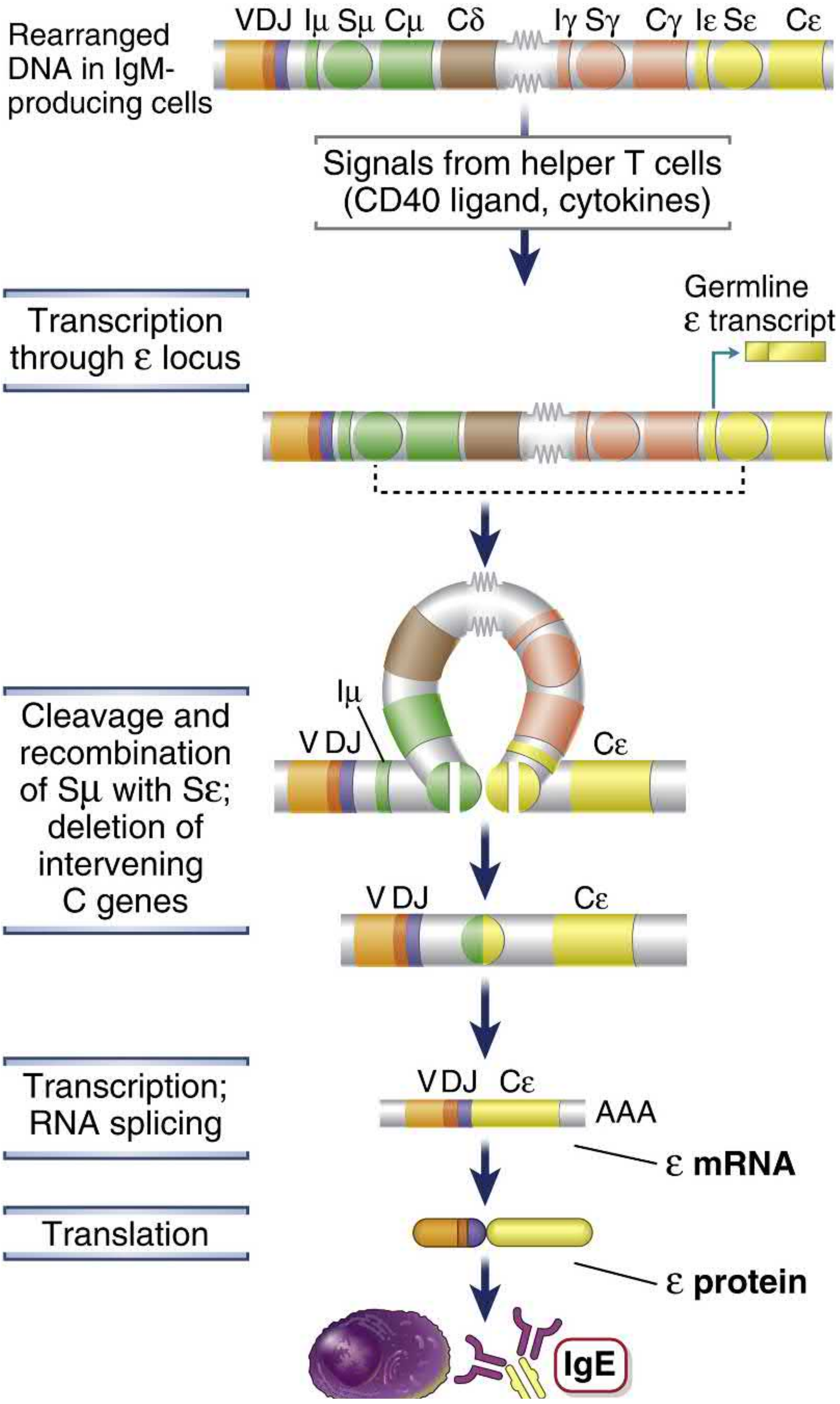
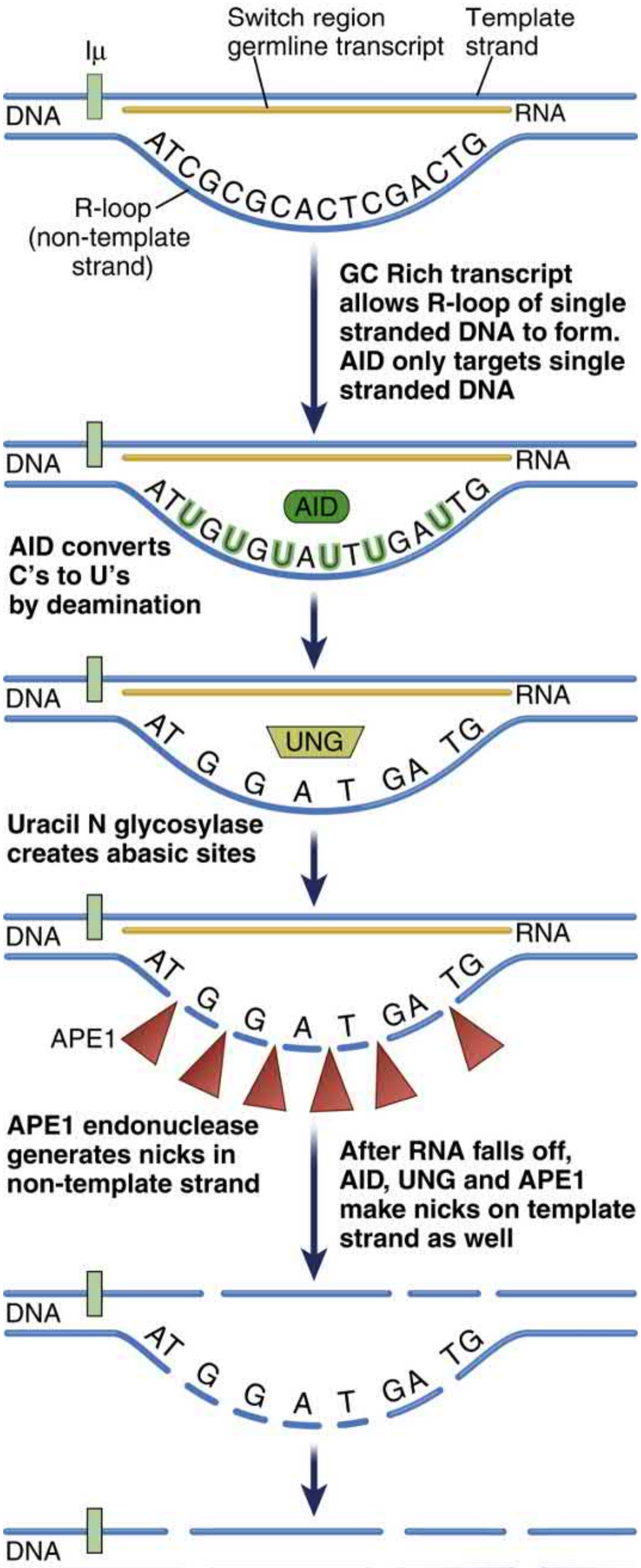

Rearranged DNA in IgM-producing cells
VDJ
Iμ
Sμ
Cμ
Cδ
Iγ
Sγ
Cγ
Iε
Sε
Cε
Signals from helper T cells (CD40 ligand, cytokines)
Transcription through ε locus
Germline ε transcript
Cleavage and recombination of Sμ with Sε; deletion of intervening C genes
Iμ
V DJ
Cε
V DJ
Cε
Transcription; RNA splicing
V DJ
Cε
AAA
ε mRNA
Translation
ε protein
IgE

FIGURE 12.14 Mechanisms of heavy chain isotype switching. When antigen-activated B cells encounter helper T cell signals (CD40L and, in this example, interleukin-4), the B cells undergo switching to immunoglobulin (Ig) isotypes other than IgM (in this example, IgE). These stimuli initiate germline transcription through the Iε-Sε-Cε locus, and the proximal C_H genes are deleted, leading to recombination of the VDJ exon upstream of the μ locus with the Cε gene. Switch regions are indicated by circles labeled Sμ, Sγ, and Sε. Iμ, Iγ, and Iε represent the initiation sites for germline transcription.

Note that there are multiple Cγ genes located between Cδ and Cε and Cα genes downstream of Cε, but these are not shown.

Cytokines determine which C_H region will undergo germline transcription. For instance, IL-4 induces germline transcription through the Iε-Sε-Cε locus (see Fig. 12.14). This leads first to the production of germline ε transcripts in an IgM-expressing B cell and then to recombination of the Sμ switch region with the Sε switch region. The intervening DNA is lost, and the VDJ exon is thus brought adjacent to Cε. The end result is the production of IgE with the same V domain as that of the original IgM produced by that B cell.

The key enzyme required for isotype switching (and somatic hypermutation, described later) is AID. As we mentioned earlier, AID expression is induced in activated B cells mainly by CD40 signals from Tfh cells. The enzyme removes an amino group from cytosines in single-stranded DNA templates, converting cytosine (C) residues to deaminated uracil (U) residues (Fig. 12.15). How AID is targeted to switch regions is poorly understood. This enzyme has a propensity for certain GC-containing tetranucleotide motifs. Switch regions are rich in these motifs, and cytokine-induced transcription through these regions makes them accessible to AID. The relative specificity of AID for switch regions, compared with other regions of the genome also containing similar motifs, can be partially explained by the fact that these GC-rich regions contribute to increased stalling of RNA polymerase II, which, when stalled, efficiently recruits AID. Switch region transcripts tend to form stable DNA-RNA hybrids involving the template strand of DNA, thus freeing up the non-template strand, which forms an open single-stranded DNA loop called an R-loop. The generation of single-stranded DNA by R-loop formation is critical because AID can target only single-stranded DNA. The R-loop is therefore a region where a large number of C residues in the switch DNA sequence are converted to U residues by AID. An enzyme called uracil N-glycosylase (UNG) removes the U residues, leaving abasic sites. An endonuclease called APE1 cleaves these abasic sites, generating a nick at each position. Whereas R loops facilitate the formation of discontinuities in the non-template strand of DNA, a break in double-stranded DNA requires that nicks also be generated on the opposite template strand of DNA. The GC-rich switch region RNA that remains tightly bound to the template strand DNA is degraded by a protein complex called the RNA exosome, thus exposing C residues transiently on the template strand and allowing AID, UNG, and APE1 to generate some nicks on this strand as well. Nicks that are generated on both strands contribute to double-strand breaks both in the Sμ “donor” switch region and in the downstream “acceptor” switch region that is involved in a particular isotype switch event. The double-strand breaks in the two switch regions are joined together (ligated) by use of the machinery involved in double-strand DNA break repair by nonhomologous end joining. In this process, the DNA between the two switch regions is deleted, and the net result is that the original rearranged V region DNA is fused to a new constant region.


Iμ
Switch region germline transcript
Template strand
DNA
RNA
ATCGCGCACTCGACTG
R-loop (non-template strand)
DNA
RNA
AID
ATUGUGUAUTUGAUTG
DNA
RNA
UNG
AT G G A T GA TG
DNA
RNA
AT G G A T GA TG
APE1
DNA
AT G G A T GA TG
DNA

GC Rich transcript allows R-loop of single stranded DNA to form. AID only targets single stranded DNA

AID converts C's to U's by deamination

Uracil N glycosylase creates abasic sites

APE1 endonuclease generates nicks in non-template strand

After RNA falls off, AID, UNG and APE1 make nicks on template strand as well

Multiple double strand breaks in switch region

FIGURE 12.15 Mechanism by which activation-induced deaminase generates double-stranded breaks at switch regions.Germline transcripts form DNA-RNA hybrids in the switch region and activation-induced cytidine deaminase *(AID)* deaminates C residues to generate U residues in single-stranded DNA. Uracil N-glycosylase *(UNG)* removes U residues to generate abasic sites where the *APEI* endonuclease creates nicks that lead to a double-stranded break. Whereas this figure illustrates only the generation of a double-strand break in the μ switch region, a similar double-strand break occurs around the same time in the switch region for a downstream isotype, thus facilitating switch recombination and isotype switching.

Affinity Maturation: Somatic Mutation of Immunoglobulin Genes and Selection of High-Affinity B Cells

Affinity maturation is the process that leads to increased affinity of antibodies for a particular antigen as a T-dependent humoral response progresses, and it is the result of somatic mutation of Ig genes followed by selective survival of the B cells that produce antibodies with the highest affinities. The process of affinity maturation generates antibodies with an increased ability to bind antigens and thus to more efficiently neutralize and eliminate microbes and their toxins (Fig. 12.16). Helper T cells and CD40-CD40L interactions are required for somatic mutation to be initiated, and, as a result, affinity maturation is observed only in antibody responses to T-dependent protein antigens.

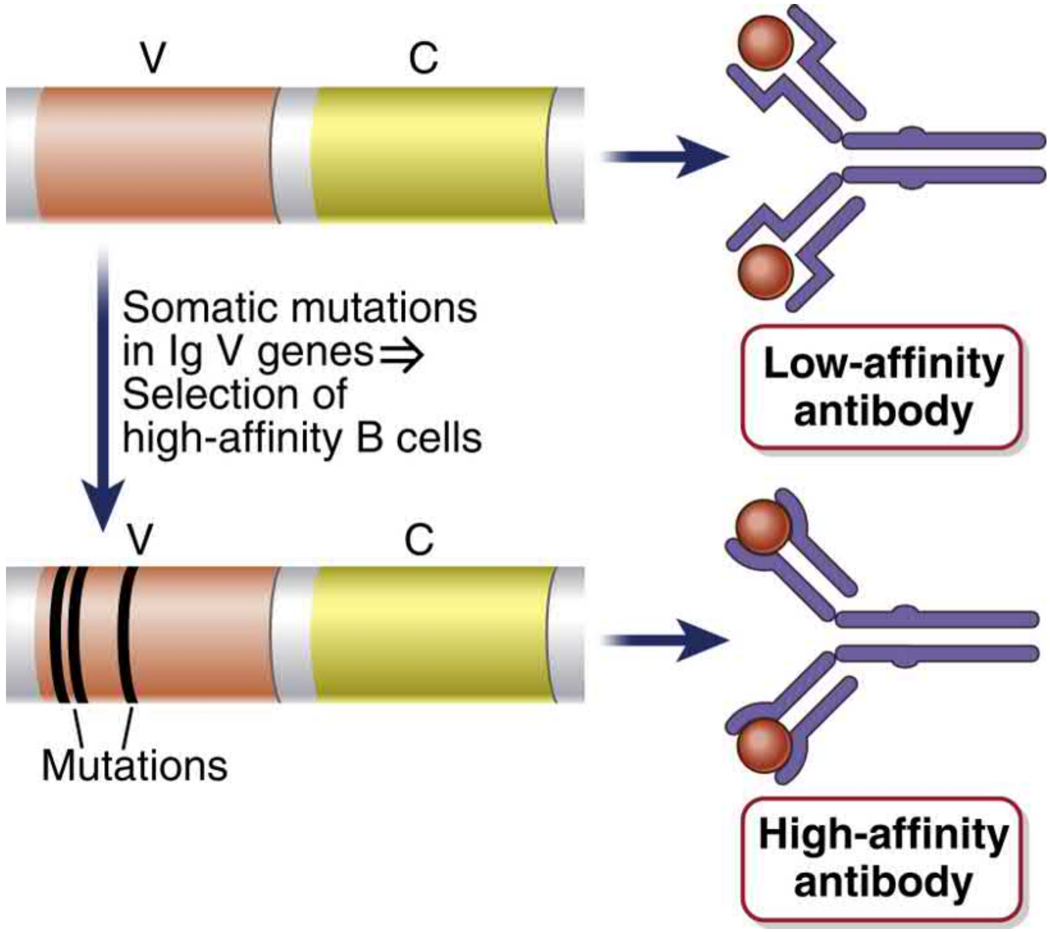

V
C
Somatic mutations in Ig V genes⇒ Selection of high-affinity B cells
Low-affinity antibody
V
C
Mutations
High-affinity antibody

FIGURE 12.16 An overview of affinity maturation. Early in the immune response, low-affinity antibodies are produced. During the germinal center reaction, somatic mutation of immunoglobulin *(Ig)* V genes and selection of B cells with high-affinity antigen receptors result in the production of antibodies with high affinity for antigen.

In proliferating germinal center B cells in the dark zone, rearranged Ig V genes undergo point mutations at an extremely high rate. This rate is estimated to be 1 in 10^3 V gene base pairs per cell division, which is approximately 1000 times higher than the spontaneous rate of mutation in other mammalian genes. For this reason, mutation in rearranged Ig V genes is also called **somatic hypermutation.** The V genes of expressed heavy and light chains in each B cell contain a total of approximately 700 nucleotides; thus, mutations will accumulate in expressed V regions at an average rate of almost one per cell division. Ig V gene mutations continue to occur in the progeny of individual B cells. As a result, any B cell clone can accumulate more and more mutations during its life in the germinal center. It is estimated that as a consequence of somatic mutations, the nucleotide sequences of IgG antibodies derived from one clone of B cells can diverge as much as 5% from the original germline sequence. This usually translates to up to 10 amino acid substitutions. The mutations are clustered in the V regions, mostly in the antigen-binding complementarity-determining regions (CDRs) (Fig. 12.17), and the presence of mutations correlates with increasing affinities of the antibodies for the antigen that induced the response.

The enzyme AID, discussed earlier in the context of isotype switching, also plays an essential role in affinity maturation. The DNA deaminase activity of AID converts C residues to U residues at specific tetranucleotide (AGCT) hotspots that are found all over the genome but are targeted primarily in rearranged V regions (or in switch regions as discussed above). AID may recognize sequences in the location of the rearranged VDJ exon, which explains at least partially why rearranged V regions are highly susceptible to mutations. However, the mechanism by which these rearranged VDJ exons are specifically targeted is still unclear. The Us that are generated from Cs may be changed to Ts when DNA replication occurs, thus generating a common type of C to T mutation, or the U may be excised by UNG, and the abasic site thus generated is repaired by an error-prone DNA repair process, eventually generating substitutions with any of the four DNA nucleotides at each site of AID-induced cytidine deamination. Two enzymes, MSH2 and MSH6, involved normally in the process of DNA mismatch repair, are important participants in somatic hypermutation. MSH2 and MSH6 can recruit nucleases that remove not only uridine nucleotides (which are normally present in RNA) but also adjacent nucleotides. This mutated stretch is then repaired by an error-prone DNA polymerase, thus extending mutations to residues beyond the C residues that are targeted by AID. It is unclear how two well-known DNA repair mechanisms, base excision repair and mismatch repair, which are normally high-fidelity processes, recruit error prone DNA polymerases in germinal center B cells in the context of somatic hypermutation.

Repeated stimulation by T cell–dependent protein antigens (in the light zone, as described later) leads to the migration of B cells into the dark zone and an increasing numbers of mutations in the Ig genes of antigen-specific germinal center B cells. Some of these mutations are likely to be useful because they will generate high-affinity antibodies. However, many of the mutations may result in a decline or even a loss of antigen binding. Therefore, the next and crucial step in the process of affinity maturation is the selection of the most useful, high-affinity B cells, a type of Darwinian natural selection that ensures survival of the best B cells (fittest in terms of antigen binding), and this occurs in the light zone.

B cells that bind antigens in germinal centers with high affinity are selected to survive (Fig. 12.18) . The early response to antigen results in the production of antibodies, some of which form complexes with residual antigen and may activate complement. FDCs express receptors for the Fc portions of antibodies and for products of complement activation, including C3b and C3d. These receptors bind and display antigens that are complexed with antibodies and complement products. Antigen also may be displayed in free form in the germinal center. Meanwhile, germinal center B cells that have undergone somatic mutation migrate into the FDC-rich light zone of the germinal center. These B cells die by apoptosis unless they are rescued by recognition of antigen. Only B cells with high-affinity receptors for the antigen are able to bind the antigen when it is present at low concentrations, and these B cells survive preferentially because of several mechanisms. First, antigen recognition by itself induces expression of anti-apoptotic proteins of the BCL-2 family. Second, high-affinity B cells will preferentially endocytose and present the antigen and interact with the limiting numbers of Tfh cells in the germinal center. These Tfh cells may signal via CD40L and cytokines to promote the survival of the B cells with which they interact.

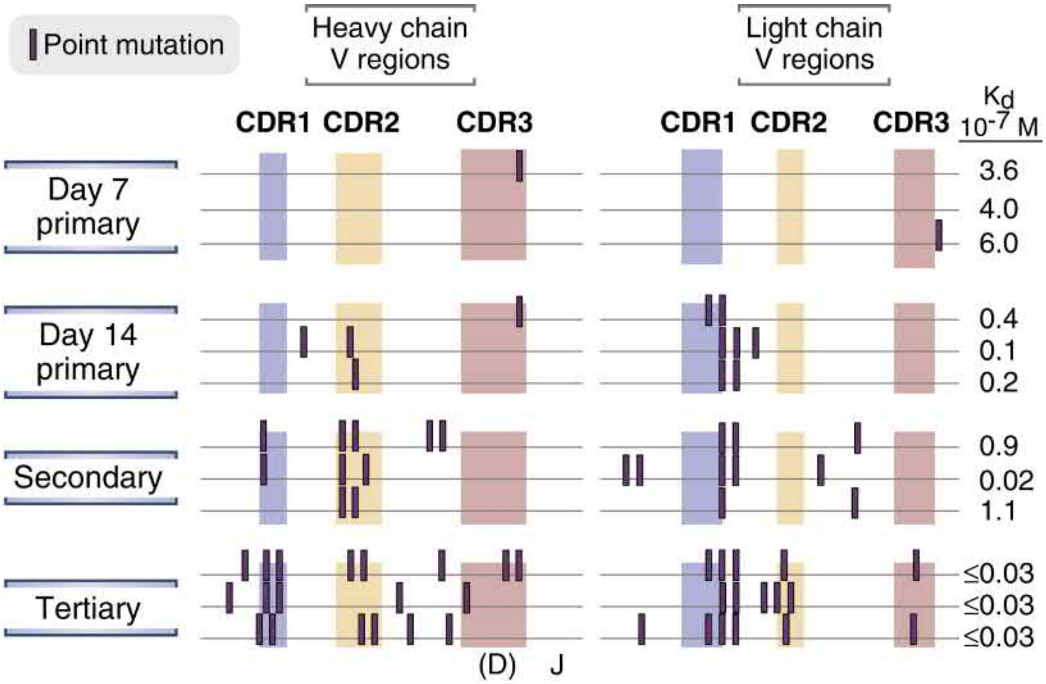

Point mutation
Heavy chain V regions
Light chain V regions
CDR1 CDR2 CDR3
CDR1 CDR2 CDR3
Kd 10-7 M
Day 7 primary
3.6
4.0
6.0
Day 14 primary
0.4
0.1
0.2
Secondary
0.9
0.02
1.1
Tertiary
≤0.03
≤0.03
≤0.03
(D) J

FIGURE 12.17 Somatic mutations in immunoglobulin V genes. Hybridomas were produced from spleen cells of mice immunized 7 or 14 days previously with a hapten, oxazolone, coupled to a protein and from spleen cells obtained after secondary and tertiary immunizations with the same antigen. Hybridomas producing oxazolone-specific monoclonal antibodies were produced, and the nucleotide sequences of the V genes encoding the immunoglobulin *(Ig)* heavy and light chains were determined. Mutations in V genes increase with time after immunization and with repeated immunizations and are clustered in the complementarity-determining regions (CDRs). The location of CDR3 in the heavy chains is approximate. The affinities of the antibodies produced also tend to increase with more mutations, as indicated by the lower dissociation constants (K_d) for hapten binding.

Modified from Berek C, Milstein C. Mutation drift and repertoire shift in maturation of the immune response. *Immunol Rev.* 1987;96:23–41.

As more antibody is produced, more of the antigen is eliminated and less is available in the germinal centers. Therefore, the B cells that will be able to specifically bind this antigen and to be rescued from death need to express antigen receptors with higher and higher affinity for the antigen. As a result, as the antibody response to an antigen progresses, the B cells that are selected to survive in germinal centers produce Ig of increasing affinity for the antigen. This selection process results in affinity maturation of the antibody response. Because somatic mutation also generates many B cells that do not express high-affinity receptors for antigen and cannot therefore be selected to survive, the germinal centers are sites of tremendous apoptotic death of B cells.

The DNA breaks associated with somatic hypermutation and isotype switching predispose to chromosomal translocations of various oncogenes into Ig gene loci, producing tumors of B cells (lymphomas). This explains why many lymphomas develop from germinal center B cells. Germinal centers may also contribute to the pathogenesis of autoimmunity if somatic mutation drives a B cell clone in the germinal center to become strongly self-reactive. The response of somatically mutated B cells to self antigens is controlled by regulatory T cells, some of which express CXCR5, enter follicles, and are known as **T follicular regulatory cells.** It is possible that a deficiency of these regulatory T cells leads to increased Tfh cell numbers and survival of self-reactive B cells, resulting in autoimmunity.

B Cell Differentiation Into Antibody-Secreting Plasma Cells

Plasma cells are morphologically distinct, terminally differentiated B cells committed to abundant antibody production (see Chapter 2) **.** They are generated after the activation of B cells through signals from the BCR, CD40, TLRs, and other receptors including cytokine receptors.

There are two types of plasma cells.

- **Short-lived plasma cells** are generated during T-independent responses and early during T-dependent responses in extrafollicular B cell foci, described earlier. These cells are generally found in secondary lymphoid organs and in peripheral nonlymphoid tissues.
- **Long-lived plasma cells** are generated in T-dependent germinal center responses to protein antigens. Signals from the B cell antigen receptor and IL-21 cooperate in the generation of plasma cells and their precursors, called **plasmablasts.** Plasmablasts are the earliest cells in the lineage of antibody-secreting cells. They continue to proliferate (like activated B cells) but express little or no CD20, the marker of mature B cells. Plasmablasts generated in germinal centers enter the circulation and home to the bone marrow, where they stop dividing and differentiate into long-lived plasma cells. These plasma cells are maintained by cytokines of the BAFF family that bind to a plasma cell membrane receptor called BCMA, which provides signals that allow the cells to survive for long periods. Typically 2 to 3 weeks after immunization with a T cell–dependent protein antigen, the bone marrow becomes a major site of antibody production. Plasma cells in the bone marrow may continue to secrete antibodies for decades after the antigen is no longer present. These antibodies can provide immediate protection if the antigen is encountered later. It is estimated that almost half the antibody in the blood of a healthy adult is produced by long-lived plasma cells and is specific for antigens that were encountered in the past. Secreted antibodies enter the circulation and mucosal secretions, but mature plasma cells do not recirculate.

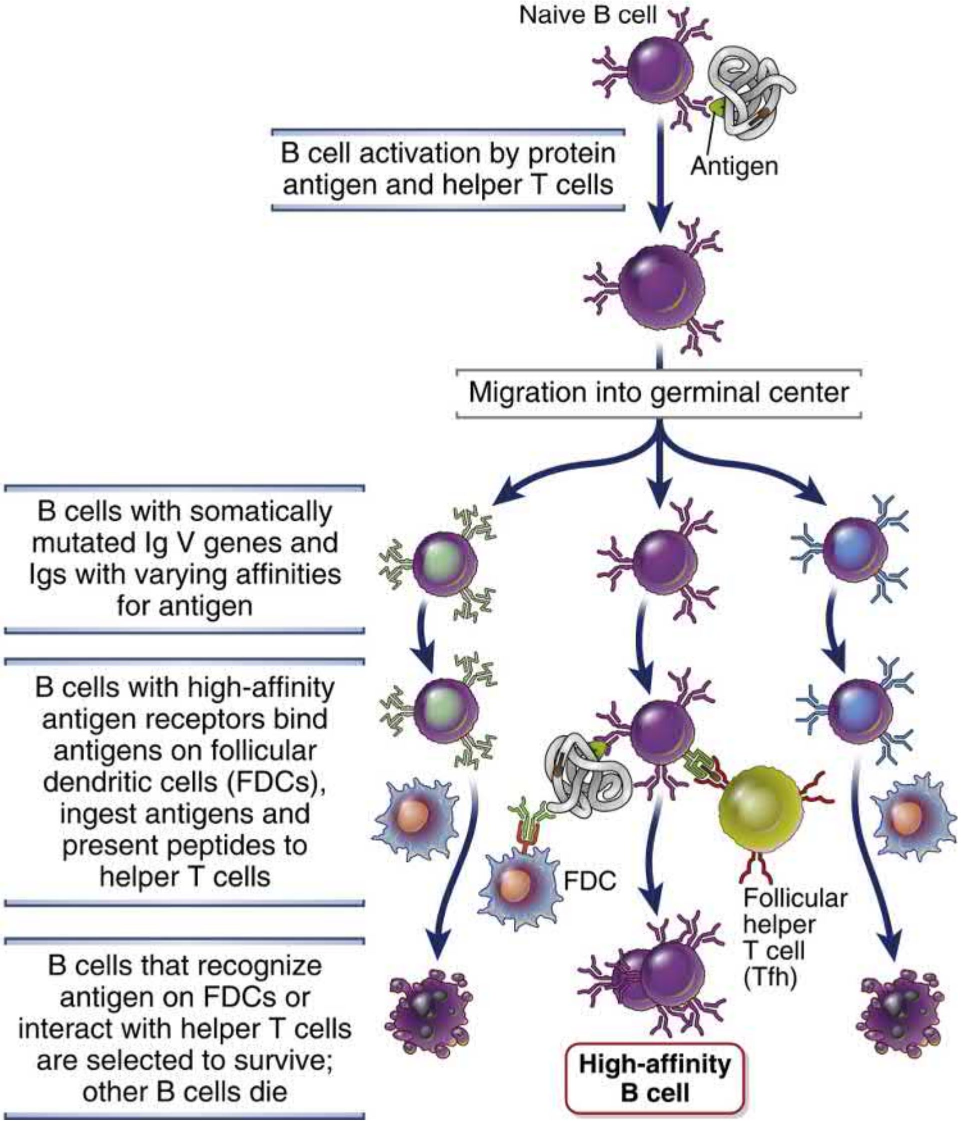

Naive B cell
Antigen
B cell activation by protein antigen and helper T cells
Migration into germinal center
B cells with somatically mutated Ig V genes and Igs with varying affinities for antigen
B cells with high-affinity antigen receptors bind antigens on follicular dendritic cells (FDCs), ingest antigens and present peptides to helper T cells
FDC
Follicular helper T cell (Tfh)
B cells that recognize antigen on FDCs or interact with helper T cells are selected to survive; other B cells die
High-affinity B cell

FIGURE 12.18 B cell selection in germinal centers.Somatic mutation of V genes in germinal center B cells generates antibodies with different affinities for antigen. Binding of the B cells to antigen displayed on follicular dendritic cells is necessary to rescue the B cells from programmed cell death. B cells may also present antigen to germinal center T follicular helper *(Tfh)* cells, which promote B cell survival. The B cells with the highest affinity for antigen thus have a selective advantage for survival as the amount of

available antigen decreases during an immune response. This leads to an average increase in the affinity of antibodies for antigen as the humoral immune response progresses.

The differentiation of B cells into antibody-secreting plasma cells involves major structural alterations in components of the endoplasmic reticulum and secretory pathway and increased Ig production as well as a change in Ig heavy chains from the membrane to the secreted form. The cell enlarges dramatically, the endoplasmic reticulum and Golgi complex become prominent (see Fig. 2.11), and the cell is transformed into a secretory cell that bears little or no resemblance to a B lymphocyte.

Ig production changes from the membrane form (characteristic of B cells) to the secreted form (in plasma cells) because of a change in the carboxy terminal of the Ig heavy chain protein (Fig. 12.19). For instance, in membrane μ, Cμ4 is followed by a short spacer, 26 hydrophobic residues, and a cytoplasmic tail of three amino acids (lysine, valine, and lysine). In secreted IgM, on the other hand, the Cμ4 domain is followed by a tail piece containing polar amino acids. This transition from membrane to secreted Ig is caused by alternative RNA processing of the heavy chain messenger RNA (mRNA). The primary RNA transcript in all IgM-producing B cells contains the rearranged VDJ cassette, the four Cμ exons coding for the constant (C) region domains, and the two exons encoding the transmembrane and cytoplasmic domains. Alternative processing of this transcript, which is regulated by RNA cleavage and the choice of polyadenylation sites, determines whether or not the transmembrane and cytoplasmic exons are included in the mature mRNA. If they are included, the μ chain produced contains the amino acids that make up the transmembrane and cytoplasmic segments and is therefore anchored in the lipid bilayer of the plasma membrane. If, on the other hand, the transmembrane segment is excluded from the μ chain, the carboxy terminus consists of approximately 20 amino acids constituting the tail piece. Because this protein does not have a stretch of hydrophobic amino acids or a positively charged cytoplasmic tail, it cannot remain anchored in the endoplasmic reticulum membrane and is secreted. Thus, each B cell can synthesize both membrane and secreted Ig. Most of the Ig heavy chain mRNA in a plasma cell is cleaved at the upstream polyadenylation site, so most of this mRNA is of the secretory form. It is not known why differentiation of B cells to plasma cells is associated with altered splicing of the Ig RNA. All C_H genes contain similar transmembrane exons, and all heavy chains can be potentially expressed in membrane-bound and secreted forms.

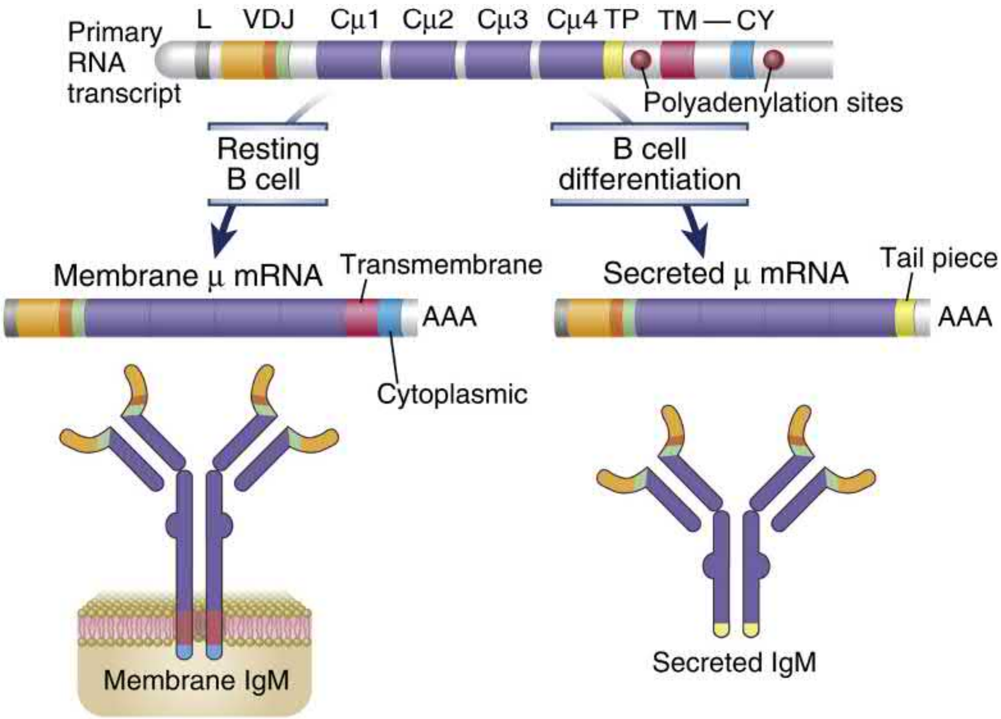

Primary RNA transcript
L
VDJ
Cμ1
Cμ2
Cμ3
Cμ4
TP
TM
CY
Polyadenylation sites
Resting B cell
B cell differentiation
Membrane μ mRNA
Transmembrane
AAA
Cytoplasmic
Secreted μ mRNA
Tail piece
AAA
Membrane IgM
Secreted IgM

FIGURE 12.19 Production of membrane and secreted **μ** chains in B lymphocytes. Alternative processing of a primary RNA transcript results in the formation of mRNA for the membrane or secreted form of the μ heavy chain. B cell differentiation results in an increasing fraction of the μ protein produced as the secreted form. *TP, TM,* and *CY* refer to tail piece, transmembrane, and cytoplasmic segments, respectively, and *AAA* refers to polyadenylation. Cμ1, Cμ2, Cμ3, and Cμ4 are four exons of the Cμ gene. *IgM,* Immunoglobulin M.

Generation of Memory B Cells

Memory B cells are generated during the germinal center reaction and are capable of making rapid responses to subsequent introduction of antigen. Because memory cells develop mainly in germinal centers, they are primarily generated during T-dependent immune responses. Although the majority of memory B cells develop in germinal centers in a T-dependent manner, some IgM-expressing memory B cells are generated without T cell help and with little or no somatic hypermutation in a T-independent manner. Memory cells survive for long periods, apparently without continuing antigenic stimulation, because they express high levels of the anti-apoptotic protein BCL-2. Some memory B cells may remain in the lymphoid organ where they were generated, whereas others exit germinal centers and recirculate between the blood and lymphoid organs. Memory cells develop from lower affinity antigen-specific B cells that emerge from the germinal center relatively early after limited rounds of somatic hypermutation and selection. The production of large quantities of isotype-switched, high-affinity antibodies is greatly accelerated after secondary exposure to antigens, and this can be attributed to the activation of memory cells. Many of the features of secondary antibody responses to protein antigens, and their differences from primary responses (see Fig. 12.3), reflect the differences between responses of memory cells and naive B cells, respectively.

Effective vaccines against microbes and microbial toxins must induce both long-lived plasma cells producing high-affinity antibodies and memory B cells, and these will occur only if the vaccines are able to activate helper T cells. This concept has been applied to the design of vaccines for some bacterial infections in which the target antigen is a capsular polysaccharide, which is incapable of stimulating T cells. In these cases, the polysaccharide is covalently linked to a foreign protein to form the equivalent of a hapten-carrier conjugate, which does activate helper T cells. Such vaccines, which are called **conjugate vaccines,** more readily induce high-affinity antibodies and memory cells than do polysaccharide vaccines without linked proteins (see Fig. 16.13). Conjugate vaccines have proved particularly effective at inducing protective immunity in infants and young children, who are less able to make strong T-independent responses to polysaccharides than are adults.

TABLE 12.3

	Thymus-Dependent Antigen	Thymus-Independent Antigen
Chemical nature	Proteins	Polymeric antigens, especially polysaccharides; also glycolipids, nucleic acids
Features of Antibody Response		
Isotype switching	Yes; IgG, IgE, and IgA	Low levels of IgG and IgA
Affinity maturation	Yes	No
Secondary response (memory B cells)	Yes	Less; only seen with some polysaccharides

Role of Transcriptional Regulators in Determining the Fate of Activated B Cells

The outcome of B cell differentiation is regulated by the induction and activation of different transcription factors. It is clear from the discussion so far that activated B cells can follow several fates. They can develop into short-lived or long-lived plasma cells, which secrete large amounts of antibodies, or into long-lived memory cells, which do not secrete antibodies but survive for prolonged periods and respond rapidly to antigen challenge. In Chapter 10, we discussed the concept that T cell fates are determined in large part by the expression of various transcriptional activators and repressors. The same general principle applies to the fates of activated B cells. The major transcription factors involved in determining the fate of germinal center B cells are the following:

- ***BCL-6.*** In germinal center B cells, signals delivered through CD40 and the IL-21 receptor induce the expression of BCL-6, which functions as a transcriptional repressor to maintain the germinal center reaction, particularly the massive proliferation of germinal center B cells. BCL-6 represses the expression of cyclin-dependent kinase inhibitors and thus cooperates with transcriptional activators, such as c-MYB, to orchestrate rapid cell cycle entry of germinal center B cells. BCL-6 also represses p53, a transcription factor that mediates cell cycle arrest and apoptotic cell death after DNA damage. As a result, dark zone B cells can tolerate the

DNA breaks that accompany isotype switching and somatic hypermutation and are relatively resistant to apoptosis. BCL-6 antagonizes another transcriptional repressor called B lymphocyte–induced maturation protein 1 (BLIMP1), which is required for plasma cell development (see later), and thus prevents cells in the germinal center from prematurely differentiating into plasma cells during the germinal center reaction.

- ***BLIMP1 and IRF4.*** BLIMP1, a transcriptional repressor, and IRF4, a transcriptional activator, are induced in some of the activated B cells and commit these cells to a plasma cell fate. In addition to suppressing BCL-6, the repressor that maintains the germinal center B cell reaction, BLIMP1 suppresses a second transcription factor, PAX5, which is required for the maintenance of mature B cells. Thus, BLIMP1 is permissive for plasma cell development. IRF4 contributes to the expression of XBP1, a transcription factor that plays a critical role in the unfolded protein response. XBP1 protects developing plasma cells from the injurious consequences of unfolded proteins (which are produced as a consequence of the massive increase in protein synthesis) and contributes to the maturation of plasma cells and the enhanced synthesis of Ig seen in these cells.
- ***HHEX.*** Relatively low-affinity B cells that receive limited T cell help in the light zone downregulate BCL-6, fail to induce MYC, have reduced mTORC activation, and differentiate into memory B cells. Memory B cells require the HHEX transcription factor that is induced in the absence of BCL-6, and it in turn induces and maintains the level of the anti-apoptotic protein BCL-2, which is required for the survival of memory B cells.
- ***Other transcription factors.*** Numerous other transcription factors are known to contribute to germinal center B cell induction and maintenance. An important regulator of the germinal center B cell program that represses other fates is BACH2. The transcription factor FOXO1 is required for the

development of dark zone germinal center B cells. It induces the expression of CXCR4, which is required for migration to the dark zone. In the light zone, PI-3 kinase signaling downregulates FOXO1. When B cells are positively selected in the light zone, they express MYC and FOXO1 and migrate to the dark zone.

Antibody Responses to T-Independent Antigens

Many nonprotein antigens, such as polysaccharides, lipids, and nucleic acids, stimulate antibody production in the absence of helper T cells, and these antigens and the responses they elicit are termed thymus independent (TI). These antibody responses differ in several respects from responses to T cell–dependent protein antigens (Table 12.3). The antibodies that are produced in the absence of T cell help are generally of low affinity and consist mainly of IgM, with limited isotype switching to some IgG subtypes and also to IgA.

Subsets of B Cells That Respond to T-Independent Antigens

The marginal zone and B-1 subsets of B cells are especially important for antibody responses to TI antigens. Whereas responses to T-dependent protein antigens are largely mediated by follicular B cells, other B cell subsets may be the primary responders to TI antigens (see Fig. 12.3). Marginal zone B cells are a distinct population of B cells that mainly respond to polysaccharides. After activation, these cells differentiate into short-lived plasma cells that produce mainly IgM. B-1 cells represent another lineage of B cells that responds readily to TI antigens mainly in the peritoneum and in mucosal sites.

T-independent antibody responses are initiated mainly in the spleen, peritoneal cavity, and mucosal sites. Macrophages located in the marginal zones surrounding lymphoid follicles in the spleen are particularly efficient at trapping polysaccharides when these antigens are injected intravenously. TI antigens may persist for prolonged periods on the surfaces of marginal zone macrophages, where they are recognized by specific B cells.

Mechanisms of T-Independent Antibody Responses

T-independent antigens are capable of stimulating B cell proliferation and differentiation in the absence of T cell help. The most important TI antigens, polysaccharides, glycolipids, and nucleic acids, cannot be processed and presented in association with MHC molecules, and therefore they cannot be recognized by $CD4^+$ helper T cells. Most TI antigens are multivalent, being composed of repeated identical antigenic epitopes. Such multivalent antigens may cross-link many BCR molecules on specific B cells, leading to activation without a requirement for cognate T cell help. In addition, many polysaccharides activate the complement system by the alternative or lectin pathway, generating C3d, which binds to the antigen and is recognized by CR2, thus augmenting B cell activation (see Fig. 12.5). As mentioned earlier, TI responses also may be facilitated by additional signals derived from microbial products that activate TLRs on B cells.

Although TI responses typically exhibit little isotype switching, some T-independent nonprotein antigens do induce Ig isotypes other than IgM. In humans, the dominant antibody class induced by pneumococcal capsular polysaccharide is IgG2. In mice engineered to lack CD40, IgE and many IgG subclasses are barely detectable in the serum, but low levels of IgG3 (which resembles human IgG2) and IgA in the serum were preserved. Cytokines produced by non-T cells may stimulate isotype switching in T-independent responses. As described earlier, in the absence of T cells, BAFF and APRIL produced by cells of myeloid origin, such as DCs and macrophages, can induce the synthesis of AID in antigen-activated B cells through a receptor of the BAFF receptor family called TACI. This may be further facilitated by the activation of TLRs on these B cells. In addition, cytokines such as TGF-β that help to mediate the IgA switch in B cells are secreted by many nonlymphoid cells at mucosal sites and may contribute to the generation of IgA antibodies directed against nonprotein antigens (see Chapter 14).

Protection Mediated by T-Independent Antibodies

The practical significance of TI antigens is that many bacterial cell wall polysaccharides belong to this category, and humoral immunity is the major mechanism of host defense against infections by such encapsulated bacteria. For this reason, individuals with congenital or acquired deficiencies of humoral immunity are especially susceptible to life-threatening infections with encapsulated bacteria, such as pneumococcus, meningococcus, and *Haemophilus*.

T-independent antigens also contribute to the generation of **natural antibodies,** which are present in the circulation of normal individuals and are apparently produced without overt exposure to pathogens. Most natural antibodies are low-affinity anti-carbohydrate antibodies, postulated to be produced by peritoneal B-1 cells responding to antigens from bacteria that colonize the gastrointestinal tract and by marginal zone B cells in the spleen. A large proportion of the natural antibodies in humans and mice are specific for oxidized lipids, such as lysophosphatidylcholine and phosphorylcholine. These lipids are found on bacterial membranes and on apoptotic cells but are not exposed on the surface of healthy host cells. Some experimental evidence indicates that the natural antibodies specific for these phospholipids provide protection against bacterial infections and facilitate the phagocytosis of apoptotic cells. The anti-ABO blood group antibodies, another example of natural antibodies, recognize certain glycolipids (blood group antigens) expressed on the surface of many cell types, including blood cells (see Chapter 17). Natural antibodies specific for blood group antigens are important barriers to blood transfusion and transplantation but are not important for host defense.

Antibody Feedback: Regulation of Humoral Immune Responses By Fc Receptors

Secreted antibodies inhibit continuing B cell activation by forming antigen-antibody complexes that simultaneously bind to antigen receptors and inhibitory Fcγ receptors on antigen-specific B cells (Fig. 12.20) **.** This is the explanation for a phenomenon called antibody feedback, which refers to the downregulation of antibody production by secreted IgG antibodies. IgG antibodies inhibit B cell activation by forming complexes with the antigen, and these complexes bind to a B cell receptor for the Fc portions of the IgG, called the Fcγ receptor II (FcγRIIB, or CD32). (We will discuss Fc receptors in Chapter 13.) The cytoplasmic tail of FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) (see Chapter 7). When this Fcγ receptor is engaged, the ITIM on the cytosolic tail of the receptor is phosphorylated on tyrosine residues, and it forms a docking site for the phosphatase SHIP (SH2 domain-containing inositol phosphatase). The recruited SHIP hydrolyses a phosphate on the signaling intermediate phosphatidylinositol triphosphate (PIP3) and inactivates this molecule. By this mechanism, engagement of FcγRIIB terminates the B cell response to antigen. The antigen-antibody complexes simultaneously interact with the antigen receptor (through the antigen) and with FcγRIIB (through the antibody), and this brings the inhibitory phosphatases close to the antigen receptors whose signaling is blocked.

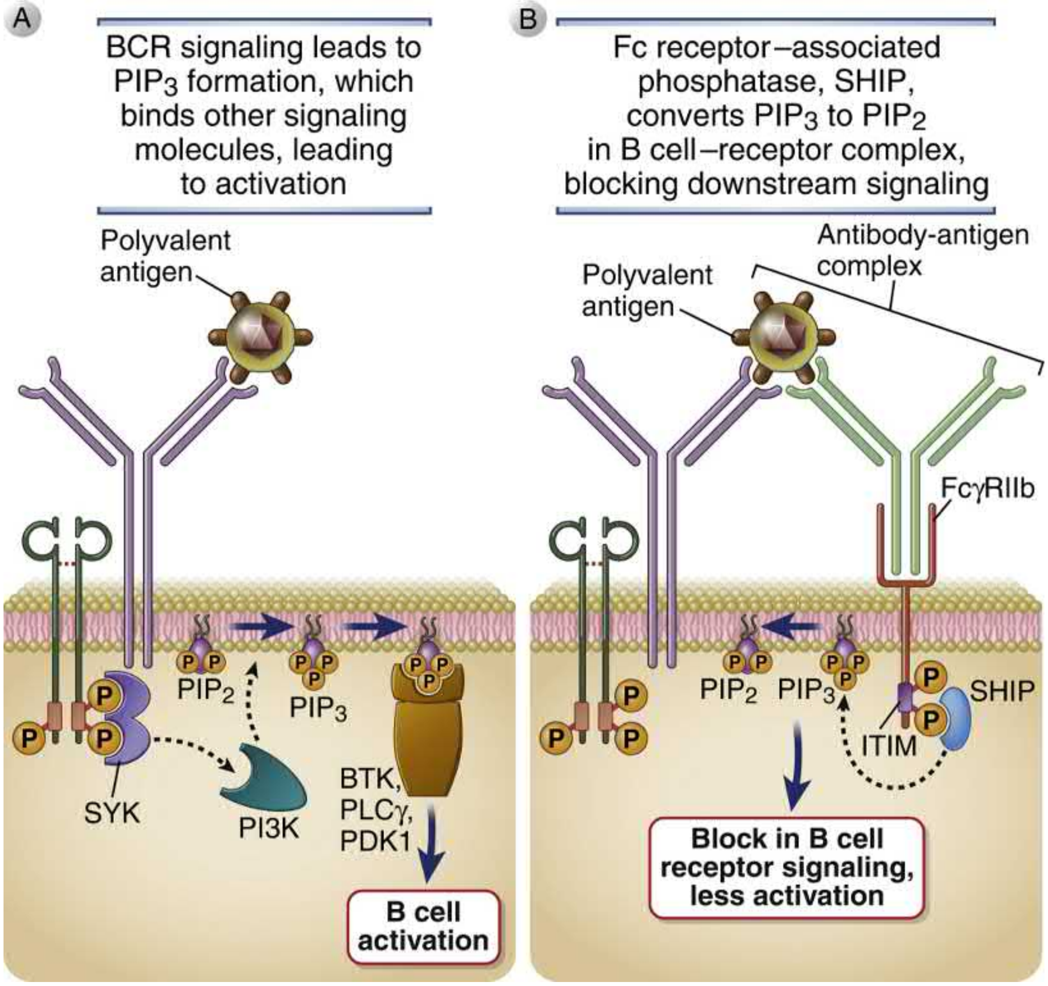

A
BCR signaling leads to PIP3 formation, which binds other signaling molecules, leading to activation
Polyvalent antigen
P
P
P
SYK
PIP2
PI3K
PIP3
BTK, PLCγ, PDK1
B cell activation
B
Fc receptor–associated phosphatase, SHIP, converts PIP3 to PIP2 in B cell–receptor complex, blocking downstream signaling
Polyvalent antigen
Antibody-antigen complex
FcγRIIb
P
P
P
PIP2
PIP3
ITIM
SHIP
Block in B cell receptor signaling, less activation

FIGURE 12.20 Regulation of B cell activation by Fc**γ**RIIB. **A,** Antigen-antibody complexes can simultaneously bind to membrane immunoglobulin (through antigen) and the FcγRIIB receptor through the Fc portion of the antibody. **B,** As a consequence of this simultaneous ligation of receptors, phosphatases associated with the cytoplasmic tail of the FcγRIIB inhibit signaling by the B cell receptor *(BCR)* complex and block B cell activation. *ITIM,* Immunoreceptor tyrosine-based inhibition motif; *PIP2,* phosphatidylinositol bisphosphate; *PIP3,* phosphatidylinositol trisphosphate; *SHIP,* SH2 domain–containing inositol phosphatase.

Fc receptor–mediated antibody feedback is a physiologic control mechanism in humoral immune responses because it is triggered by secreted antibody and blocks further antibody production. The importance of FcγRIIB-mediated inhibition is demonstrated by the uncontrolled antibody production seen in mice in which the gene encoding this receptor has been knocked out. A polymorphism in the *FcγRIIB* gene has been linked to susceptibility to the autoimmune disease systemic lupus erythematosus in humans.

B cells express another inhibitory receptor called CD22, which is a sialic acid–binding lectin; its natural ligand is not known, nor is it known exactly how CD22 is engaged during B cell responses. However, knockout mice lacking CD22 show greatly enhanced B cell activation. The cytoplasmic tail of this molecule contains ITIM tyrosine residues, which, when phosphorylated by the SRC family kinase LYN, bind the SH2 domain of the tyrosine phosphatase SHP1. SHP1 removes phosphates from the tyrosine residues of several enzymes and adaptor proteins involved in BCR signaling and thus abrogates B cell activation. A mouse strain called *motheaten,* which develops severe autoimmunity with uncontrolled B cell activation and autoantibody production, has a naturally occurring mutation in SHP1. Conditional deletion of SHP1, as well as the engineered loss of LYN in B cells, leads to a breakdown of peripheral B cell tolerance and the development of autoimmunity.

Summary

- In humoral immune responses, B lymphocytes are activated by antigen and secrete antibodies that act to eliminate the antigen. Both protein and nonprotein antigens can stimulate antibody responses. B cell responses to protein antigens require the contribution of $CD4^+$ helper T cells specific for the antigen.
- Helper T cell–dependent B cell responses to protein antigens require initial independent activation of naive T cells in the T cell zones and of B cells in lymphoid follicles

in lymphoid organs, each specific for a different part of the same protein antigen.

- A B cell that recognizes a conformational epitope of a native protein antigen internalizes the protein, processes it, and displays a peptide derived from the protein on its class II major histocompatibility (MHC) molecules for recognition by helper T cells.
- The activated lymphocytes migrate toward one another and interact at the edges of follicles, where the B cells present the peptide antigen to antigen-specific helper T cells.
- Activated helper T cells express CD40 ligand (CD40L), which engages CD40 on the B cells, and the T cells secrete cytokines that bind to cytokine receptors on the B cells. The combination of CD40 and cytokine signals stimulates B cell proliferation and differentiation.
- Stimulation of activated B cells at extrafollicular sites by helper T cells leads to the formation of extrafollicular foci where isotype switching occurs and short-lived plasma cells are generated.
- Some activated helper T cells differentiate into specialized T follicular helper (Tfh) cells that express high levels of inducible costimulator (ICOS) and CXCR5 and secrete interleukin-21 (IL-21). Tfh cells and antigen-activated B cells migrate into the follicle, and Tfh cells activate these specific B cells to initiate the formation of germinal centers. The late events in T cell–dependent antibody responses, including additional isotype switching, somatic mutation, affinity maturation, generation of memory B cells, and induction of long-lived plasma cells, take place within germinal centers.
- Helper T cell–derived signals, including CD40L and cytokines, induce isotype switching in B cells by a process of switch recombination, leading to the production of various immunoglobulin (Ig) isotypes. Isotype switching requires the induction of activation-induced deaminase (AID), a cytidine deaminase that converts cytosine to uracil

in single-stranded DNA, and different cytokines allow AID to access distinct downstream heavy chain loci.

- Affinity maturation occurs in germinal centers and leads to increased affinity of antibodies during the course of a T cell–dependent humoral response. Affinity maturation is a result of somatic mutation of Ig heavy and light chain genes induced by AID, followed by selective survival of the B cells that produce high-affinity antibodies and bind to antigen displayed by follicular dendritic cells in the germinal centers. The high-affinity B cells are best able to present antigens to Tfh cells, which promote survival of the B cells.
- Some of the progeny of germinal center B cells differentiate into antibody-secreting plasma cells that migrate to the bone marrow. Other progeny become memory B cells that live for long periods, recirculate between lymphoid organs and peripheral tissues, and respond rapidly to subsequent exposures to antigen by differentiating into high-affinity antibody secretors. The expression of various transcription factors controls the differentiation of activated B cells into plasma cells or memory cells.
- T-independent (TI) antigens are generally nonprotein antigens that induce humoral immune responses without the involvement of helper T cells. Many TI antigens, including polysaccharides, membrane glycolipids, and nucleic acids, are multivalent, can cross-link multiple membrane Ig molecules on a B cell, and activate complement, thereby activating the B cells without T cell help. Toll-like receptor (TLR) activation on B cells by microbial products may facilitate T-independent B cell activation.
- TI antigens stimulate antibody responses in which there is limited heavy chain class switching, affinity maturation, or memory B cell generation because these features are largely dependent on helper T cells, which are not activated by nonprotein antigens. However, some T-independent isotype switching can be induced by TLR stimulation by microbes,

which may lead to the production of cytokines of the TNF family that activate B cells to induce AID.

- Antibody feedback is a mechanism by which humoral immune responses are downregulated when enough antibody has been produced and soluble antibody–antigen complexes are present. B cell membrane Ig and the receptor on B cells for the Fc portions of IgG, called FcγRIIB, are clustered together by antibody-antigen complexes. This activates an inhibitory signaling cascade through the cytoplasmic tail of FcγRIIB that terminates the activation of the B cell.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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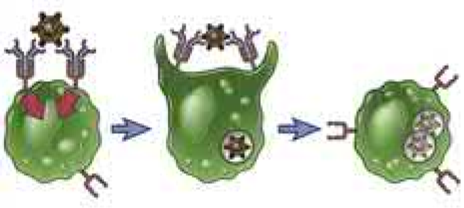
Chapter 13: Effector Mechanisms of Humoral Immunity



- Overview of Humoral Immunity,
- Neutralization of Microbes and Microbial Toxins,
- Antibody-Mediated Opsonization and Phagocytosis,
  - Leukocyte Fc Receptors,
  - Antibody-Dependent Cell-Mediated Cytotoxicity,
  - Antibody-Mediated Clearance of Helminths,
- The Complement System,
  - Pathways of Complement Activation,
  - Receptors for Complement Proteins,
  - Regulation of Complement Activation,
  - Functions of Complement,
  - Complement Deficiencies,
  - Pathologic Effects of the Complement System,
  - Evasion of Complement by Microbes,

Summary,

Humoral immunity is mediated by secreted antibodies, and its physiologic function is defense against extracellular microbes and microbial toxins. This type of immunity contrasts with cell-mediated immunity, the other effector arm of the adaptive immune system, which is mediated by T lymphocytes and functions to eradicate microbes that infect and live inside host cells (see Chapters 10 and 11). Humoral immunity is the form of adaptive immunity that can be transferred from immunized to naive individuals with serum that contains antibodies. The types of microorganisms that are combated by humoral immunity are extracellular bacteria, fungi, and even obligate intracellular microbes, such as viruses, which are targets of antibodies before they infect cells or when they are released from infected cells. Defects in antibody production result in increased susceptibility to infection with many microbes, including bacteria, fungi, and viruses. Currently used vaccines induce protection primarily by stimulating the production of antibodies (Table 13.1). Apart from their crucial protective roles, antibodies can be harmful and mediate tissue injury in allergic individuals, in certain autoimmune diseases, in blood transfusion reactions, and in transplant rejection. In this chapter, we will discuss the effector mechanisms that are used by antibodies to eliminate microbes and their toxins. The structure of antibodies is described in Chapter 5 and the process of antibody production in Chapter 12.

Overview of Humoral Immunity

Before we discuss the mechanisms by which antibodies provide protection against microbes, we will summarize some of the salient features of antibody-mediated host defense.Antibody-mediated elimination of antigens involves a number of effector mechanisms and requires the participation of various cells and secreted proteins of the immune system, including phagocytes and complement proteins (Fig. 13.1).

Antibodies are produced by plasma cells in secondary (peripheral) lymphoid organs, inflamed tissues, and bone marrow, and antibodies can perform their effector functions at sites distant from their production. Antibodies produced in the lymph nodes, spleen, and bone marrow may enter the blood and then circulate throughout the body. In mucosal organs, such as the intestine and the airways, antibodies are produced in the lamina propria (the cell-rich connective tissue space that separates the innermost layer of epithelial cells from an underlying concentric smooth muscle layer) and transported across epithelia into the lumen, where these secreted antibodies block the entry of ingested and inhaled microbes (see Chapter 14). Antibodies are also actively transported across the placenta into the circulation of the developing fetus (see Chapter 14). In disease states, antibodies may be produced in peripheral nonlymphoid tissues, at sites of infection or chronic inflammation that are sometimes called tertiary lymphoid organs. The antibodies that mediate protective immunity may be derived from short-lived or long-lived antibody-producing plasma cells. Long-lived plasma cells reside mainly in the bone marrow. In cell-mediated immunity, activated T lymphocytes are able to migrate to peripheral sites of infection and inflammation, but they are not transported into mucosal secretions or across the placenta. Therefore, antibodies are the only defense mechanism used to combat microbes in the lumens of mucosal organs and in the fetus and newborn.

TABLE 13.1

Vaccine-Induced Humoral Immunity [a]

Infectious Disease	Vaccine	Mechanism of Protective Immunity
Polio	Injected inactivated poliovirus (Salk) and oral attenuated poliovirus (Sabin)	Neutralization of virus by IgG or by mucosal IgA antibody
Tetanus, diphtheria	Toxoids (inactivated toxins)	Neutralization of toxin by systemic IgG antibody
Hepatitis A or B	Recombinant viral envelope proteins	Neutralization of virus by mucosal IgA or systemic IgG antibody
Pneumococcal pneumonia, *Haemophilus influenzae* *infections, and bacterial meningitis caused by Neisseria meningitidis*	Conjugate vaccines composed of bacterial capsular polysaccharide attached to a carrier protein	Opsonization and phagocytosis mediated by IgM and IgG antibodies, directly or secondary to complement activation

Ig, Immunoglobulin.

[a] Selected examples of vaccines that work by stimulating protective humoral immunity are listed.

Many of the effector functions of antibodies are mediated by Fc regions of immunoglobulin (Ig) molecules, and different Ig heavy chain isotypes serve distinct effector functions (Table 13.2) **.** For instance, some IgG subclasses (IgG1 and IgG3) bind to phagocyte Fc receptors and promote the phagocytosis of antibody-coated particles; IgM and some subclasses of IgG (IgG1, IgG2 to a limited extent, and IgG3 but not IgG4) activate the complement system; and IgE binds to the Fc receptors of mast cells and triggers their activation. Each of these effector mechanisms will be discussed later in this chapter. The humoral immune system is specialized in such a way that different microbes or antigen exposures stimulate B cell switching to the Ig isotypes that are best suited for combating these microbes. The major stimuli for isotype switching during the process of B cell activation are cytokines together with CD40 ligand expressed by activated helper T cells (see Chapter 12). Neutralization is the only function of antibodies that is mediated entirely by binding of antigen and does not require participation of the Ig constant regions.

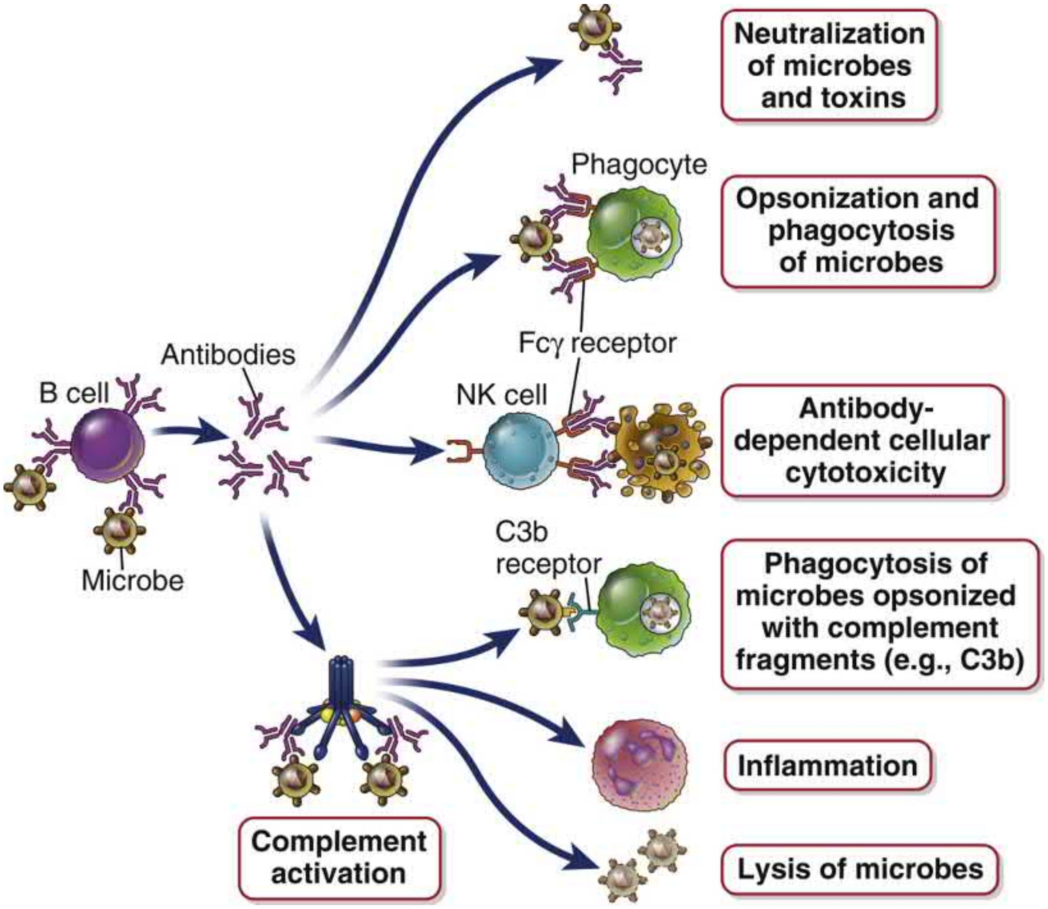

Neutralization of microbes and toxins
Phagocyte
Opsonization and phagocytosis of microbes
Fcγ receptor
Antibodies
B cell
NK cell
Antibody-dependent cellular cytotoxicity
Microbe
C3b receptor
Phagocytosis of microbes opsonized with complement fragments (e.g., C3b)
Inflammation
Complement activation
Lysis of microbes

FIGURE 13.1 Effector functions of antibodies.Antibodies against microbes (and their toxins, not shown) neutralize these agents, opsonize them for phagocytosis, sensitize them for antibody-dependent cellular cytotoxicity, and activate the complement system. These various effector functions may be mediated by different antibody isotypes. *NK,* Natural killer.

TABLE 13.2

Antibody Isotype	Effector Functions
IgG	Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells Neonatal immunity: transfer of maternal antibody across the placenta and gut Feedback inhibition of B cell activation Neutralization of microbes and toxins
IgM	Activation of the classical pathway of complement Neutralization of microbes and toxins
IgA	Mucosal immunity: secretion of IgA into the lumens of the gastrointestinal and respiratory tracts Neutralization of microbes and toxins in lumens of mucosal organs
IgE	Mast cell degranulation (immediate hypersensitivity reactions) Defense against helminths

The effector functions of antibodies that are mediated by the Fc regions are triggered by the binding of antigens to the variable regions. The binding of antibodies to a multivalent antigen, such as a polysaccharide or a repeated epitope on a microbial surface, brings multiple antibody molecules close together, and this clustering of antibody molecules leads to complement activation and allows the antibodies to bind to and activate Fc receptors on phagocytes. The requirement for antigen binding ensures that antibodies activate various effector mechanisms only when they are needed, that is, when the antibodies encounter and specifically bind antigens, not when the antibodies are circulating in an antigen-free form.

With this introduction to humoral immunity, we proceed to a discussion of the various functions of antibodies in host defense.

Neutralization of Microbes and Microbial Toxins

Antibodies against microbes and microbial toxins block the binding of these microbes and toxins to cellular receptors (Fig. 13.2) . In this way, antibodies inhibit, or neutralize, the infectivity of microbes as well as the potential injurious effects of microbial toxins. Many microbes enter host cells by the binding of particular microbial surface molecules to membrane proteins or lipids on the surface of host cells. For example, influenza viruses use their envelope hemagglutinin to infect respiratory epithelial cells, and gram-negative bacteria use pili to attach to and infect a variety of host cells. Antibodies that bind to these microbial structures interfere with the ability of the microbes to interact with cellular receptors by means of steric hindrance and thus prevent infection. Many microbial toxins mediate their pathologic effects also by binding to specific cellular receptors. For instance, tetanus toxin binds to receptors in the motor end plate of neuromuscular junctions and inhibits neuromuscular transmission, which leads to paralysis, and diphtheria toxin binds to cellular receptors and enters various cells, where it inhibits protein synthesis. Antibodies against such toxins sterically hinder the interactions of toxins with host cells and thus prevent the toxins from causing tissue injury and disease. Neutralization can occur in multiple ways that go beyond steric interference. For instance, in the lumen of the gut, aggregation or agglutination of microbes by IgA antibodies can reduce the infectivity of the pathogens, trap them in mucus, and facilitate their clearance by peristalsis. In some cases, antibodies may bind to a microbe and induce conformational changes in surface molecules that prevent the microbe from interacting with cellular receptors. Such interactions have been observed with antibodies against certain viruses and are examples of the allosteric effects of antibodies.

Antibody-mediated neutralization of microbes and toxins requires only the antigen-binding regions of the antibodies. Therefore, such neutralization may be mediated by antibodies of any isotype in the circulation and in mucosal secretions and can experimentally or therapeutically also be mediated by Fab or $F(ab)_2$ fragments of specific antibodies, which lack the Fc regions of the heavy chains. Neutralizing antibodies in the blood are mainly of the IgG isotype, and mainly IgA antibodies at mucosal sites. The most effective neutralizing antibodies are those with high affinities for their antigens. High-affinity antibodies are produced by the process of affinity maturation (see Chapter 12). Many prophylactic vaccines work by stimulating the production of high-affinity neutralizing antibodies (see Table 13.1). A mechanism that microbes have developed to evade host immunity is to mutate the genes encoding surface antigens that are the targets of neutralizing antibodies (see Chapter 16).

The role of antibodies in neutralizing microbes and toxins at particular anatomic sites, specifically in mucosal tissues and the fetus, is discussed in Chapter 14.

Antibody-Mediated Opsonization and Phagocytosis

IgG antibodies coat (opsonize) microbes and promote their phagocytosis by binding to Fc receptors on phagocytes. Mononuclear phagocytes and neutrophils ingest microbes as a prelude to intracellular killing and degradation. These phagocytes express a variety of surface receptors that directly bind microbes and ingest them, even without antibodies, which is one mechanism of innate immunity (see Chapter 4). The efficiency of this process is markedly enhanced if the phagocyte can bind the particle with high affinity. Mononuclear phagocytes and neutrophils express receptors for the Fc portions of IgG antibodies that specifically bind antibody-coated particles. Microbes may also be coated by a product of complement activation called C3b and are then phagocytosed by binding to a leukocyte receptor for C3b (described later in this chapter). As discussed in Chapter 4, the process of coating particles to promote their phagocytosis is called **opsonization,** and substances that perform this function, including antibodies, complement proteins and certain plasma lectins, are called opsonins.

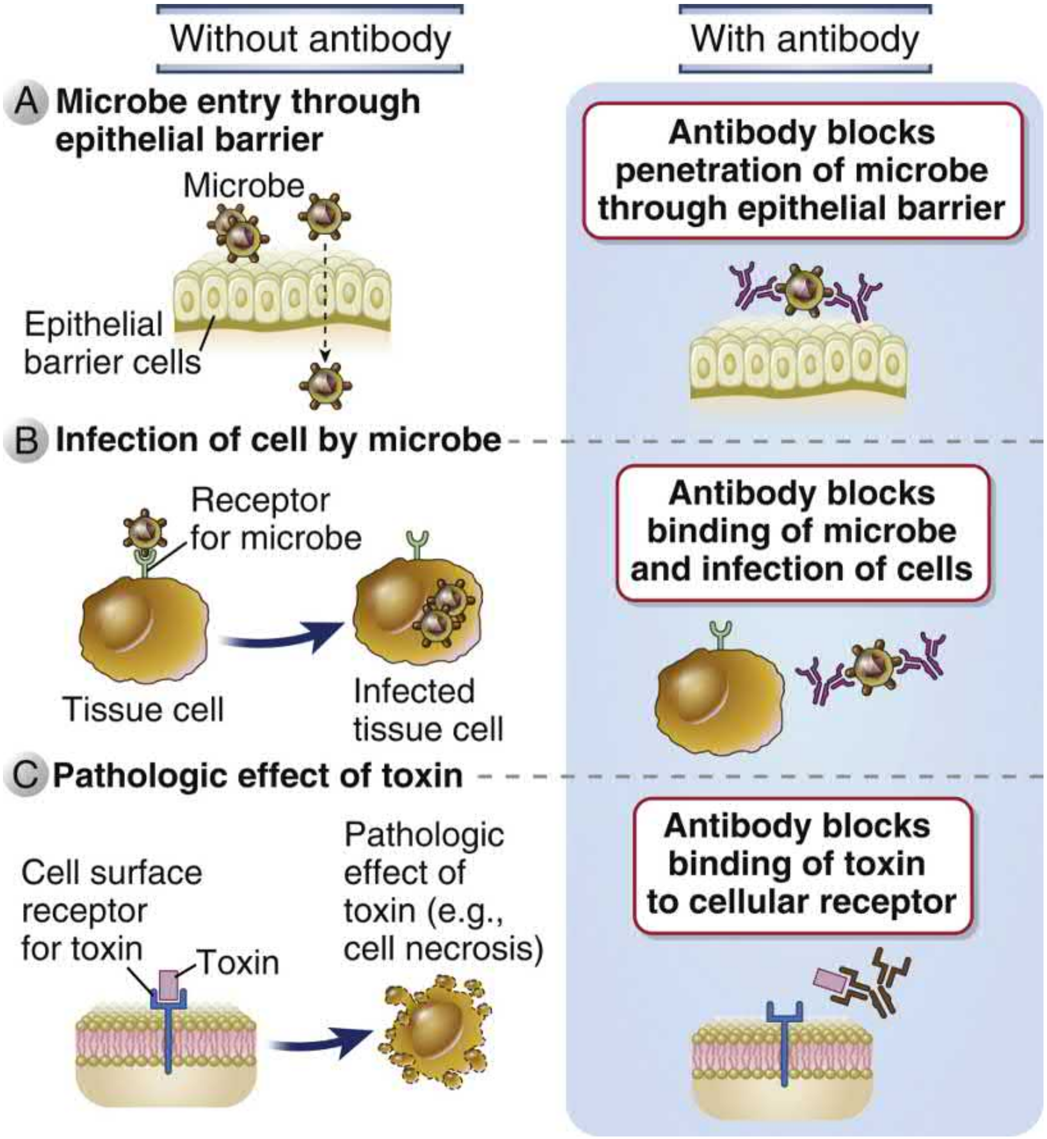

Without antibody
With antibody
A Microbe entry through epithelial barrier
Microbe
Epithelial barrier cells
Antibody blocks penetration of microbe through epithelial barrier
B Infection of cell by microbe
Receptor for microbe
Tissue cell
Infected tissue cell
Antibody blocks binding of microbe and infection of cells
C Pathologic effect of toxin
Cell surface receptor for toxin
Toxin
Pathologic effect of toxin (e.g., cell necrosis)
Antibody blocks binding of toxin to cellular receptor

FIGURE 13.2 Neutralization of microbes and toxins by antibodies. **A,** Antibodies prevent the binding of microbes to cells and thus block the ability of the microbes to infect host cells. **B,** Antibodies inhibit the spread of microbes from an infected cell to an adjacent uninfected cell. **C,** Antibodies block the binding of toxins to cells and thus inhibit the pathologic effects of the toxins.

Leukocyte Fc Receptors

Leukocytes express Fc receptors that bind to the constant regions of antibodies, and thereby promote the phagocytosis of Ig-coated particles and deliver signals that regulate the activities of the leukocytes; other Fc receptors mediate the transport of antibodies to various sites. Fc receptors for different Ig heavy chain isotypes are expressed on many leukocyte populations and serve diverse functions in immunity. Of these Fc receptors, the ones that are most important for phagocytosis of opsonized particles are specific for the heavy chains of IgG antibodies, called Fcγ receptors, and these are the receptors that will be primarily considered in this chapter. In Chapter 20, we will discuss the Fc receptors that bind IgE. In Chapter 5, we described the neonatal Fc receptor (FcRn), which is expressed in the placenta and on vascular endothelium and other cell types and has unique functions related to IgG transport across the placenta and the protection of IgG from turnover. In Chapter 14, we will discuss the poly-Ig receptor, which is involved in the transport of mainly IgA across mucosal epithelia.

Fcγ receptors have been classified into three groups, based on their affinities for heavy chains of different IgG subclasses. Different Fc receptors are also expressed on different cell types (Table 13.3). In general, IgG1- and IgG3-containing immune complexes bind efficiently to activating Fc receptors and IgG2-containing complexes do not bind well. IgG4 has a very low affinity for activating Fc receptors, and the biologic function of this antibody isotype is poorly understood. All Fc receptors are optimally activated by antibodies bound to their antigens and not by free, circulating antibodies. The binding of immune complexes to most Fc receptors results in cellular activation, except for FcγRIIB, which is an inhibitory receptor. All Fcγ receptors contain a ligand-binding chain, called the α chain, that recognizes IgG heavy chains (Fig. 13.3). Differences in specificities or affinities of each FcγR for the various IgG isotypes are based on differences in the structure of these α chains. In all of the FcRs except FcγRII, the α chain is associated with one or more additional polypeptide chains involved in signal transduction. Signaling functions of all forms of

FcγRII are mediated by the cytoplasmic tail of this single chain receptor.

TABLE 13.3

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High (K_d ~10^{-9} M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low (K_d ~10^{-7} M)	Macrophages, neutrophils, dendritic cells, eosinophils, platelets	Phagocytosis; cell activation
FcγRIIB (CD32)	Low (K_d ~10^{-7} M)	B lymphocytes, macrophages, dendritic cells, other cells	Feedback inhibition of various cellular responses
FcγRIIC (CD32)	Low (K_d ~10^{-7} M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low (K_d ~10^{-6} M)	NK cells, macrophages, dendritic cells	Antibody-dependent cell-mediated cytotoxicity (NK cells)
FcγRIIIB (CD16)	Low (K_d ~10^{-6} M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High (K_d ~10^{-10} M); binds monomeric IgE	Mast cells, basophils	Cell activation (degranulation)
FcεRII (CD23)	Low (K_d ~10^{-7} M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low (K_d ~10^{-6} M)	Neutrophils, eosinophils, monocytes	Cell activation?

GPI, Glycophosphatidylinositol; *IgG,* immunoglobulin G; *NK,* natural killer.

[a] The three groups of Fcγ receptors are numbered I, II, and III, and the isoforms in two of them are named A, B, and C.

There are three major groups of IgG-specific Fc receptors, of which two have multiple isoforms that differ in structure and function (see Table 13.3); these are described in the following list. The unique functions of the FcRn are discussed in Chapters 5 and 14.

- ***FcγRI*** (CD64) is the major phagocyte Fcγ receptor. It is expressed on macrophages and neutrophils and binds IgG1 and IgG3 with high affinity (dissociation constant [K_d] of 10^{-8} to 10^{-9} M). The large extracellular amino-terminal region of the Fc-binding *α* chain folds into three tandem Ig-like domains. The *α* chain of FcγRI is associated with a disulfide-linked homodimer of a signaling protein called the FcR γ chain. This γ chain is also found in the signaling complexes associated with FcγRIII, Fc*α*R, and FcεRI. The γ chain has only a short extracellular amino terminus but a large cytoplasmic carboxyl terminus, which is structurally homologous to the ζ chain of the T cell receptor (TCR) complex. Like the TCR ζ chain, the FcR γ chain contains an immunoreceptor tyrosine-based activation motif (ITAM) that couples receptor clustering to activation of protein tyrosine kinases. Cross-linking of several Fc receptor-bound IgG molecules by multivalent antigens results in cell activation.

- ***Fc* γ *RII*** (CD32) binds IgG1 and IgG3 with a low affinity (K_d 10^{-6} M). Gene duplication and diversification have resulted in the generation of three forms of this receptor, called FcγRII A, B, and C. These isoforms have similar extracellular domains and ligand specificities but differ in cytoplasmic tail structure, cell distribution, and functions. FcγRIIA is expressed by neutrophils, mononuclear phagocytes, and dendritic cells (DCs) and participates in the phagocytosis of opsonized particles, whereas FcγRIIC is expressed in mononuclear phagocytes, neutrophils, and natural killer (NK) cells. The cytoplasmic tails of FcγRIIA and FcγRIIC contain ITAMs which, on clustering by IgG1- or IgG3-coated particles or cells, can deliver an activation signal to phagocytes. On DCs, this receptor can contribute to capture of antigen in immune complexes and consequently T cell activation. FcγRIIB is an inhibitory receptor expressed on myeloid cells and B cells and is the only Fc receptor on B cells. Its role in antibody feedback is described in Chapter 12.
- ***FcγRIII*** (CD16) is also a low-affinity receptor for IgG. The extracellular ligand-binding portion of FcγRIII is similar to FcγRII in structure, affinity, and specificity for IgG. This receptor exists in two forms, encoded by separate genes. The FcγRIIIA isoform is a transmembrane protein expressed mainly on NK cells and also on macrophages and DCs. FcγRIIIA associates with homodimers of the FcR γ chain, homodimers of the TCR ζ chain, or heterodimers

composed of an FcR γ chain and a ζ chain. These associated chains contain ITAMs that deliver activating signals upon antibody binding to the Fc receptors and are thus necessary for the functions of the receptors. The FcγRIIIB isoform is a glycophosphatidylinositol (GPI)-linked protein expressed on neutrophils; it does not mediate phagocytosis or trigger neutrophil activation, and its function is poorly understood.

In addition to these Fcγ receptors, there are receptors for the heavy chains of IgE and IgA (see Table 13.3). We will describe FcεRI in Chapter 20. The function of FcαR is not well established.

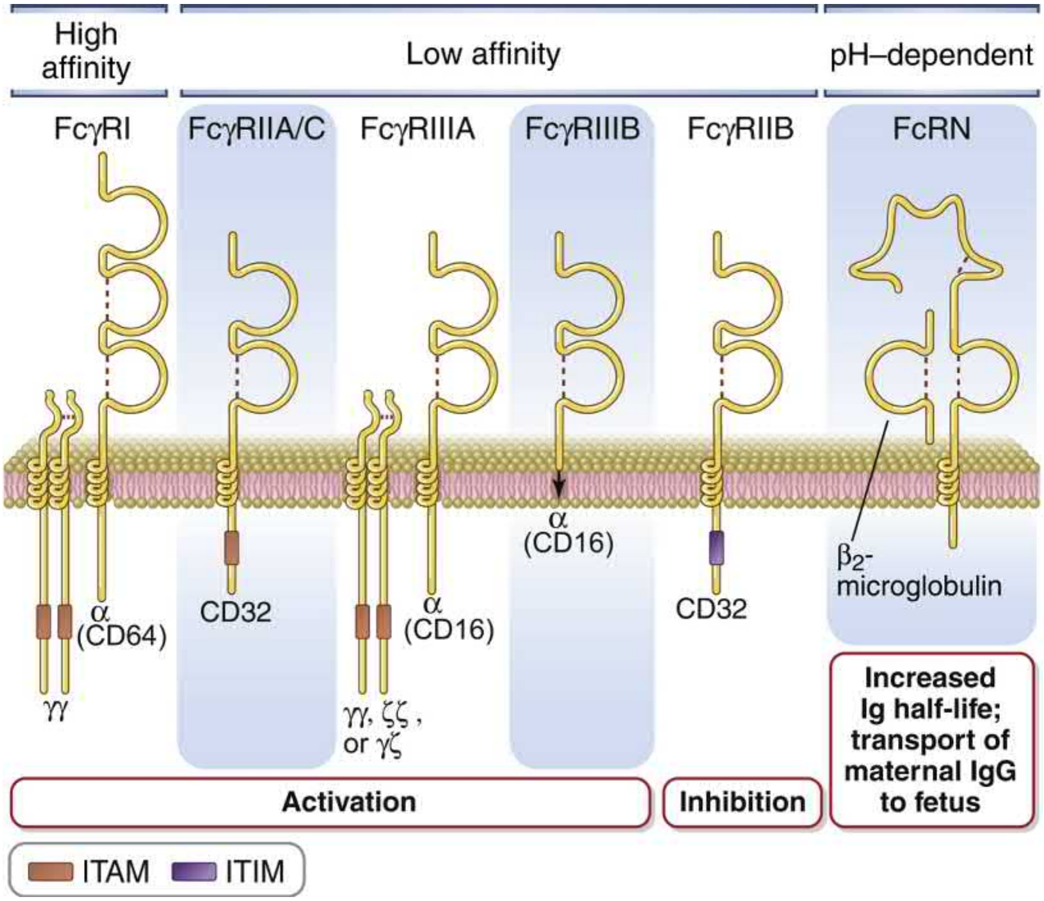

High affinity
Low affinity
pH–dependent
FcγRI
FcγRIIA/C
FcγRIIIA
FcγRIIIB
FcγRIIB
FcRN
α (CD64)
γγ
CD32
α (CD16)
γγ, ζζ, or γζ
α (CD16)
CD32
β2-microglobulin
Activation
Inhibition
Increased Ig half-life; transport of maternal IgG to fetus
ITAM
ITIM

FIGURE 13.3 Subunit composition of Fcγ receptors.Schematic models of the different human Fc receptors illustrate the Fc-binding α chains and the signaling subunits. FcγRIIIB is a glycophosphatidylinositol (GPI)-anchored membrane protein with no known signaling functions. FcγRIIA and IIC are structurally similar low-affinity activating receptors with slightly different patterns of expression. Note that although FcγRIIA/C and FcγRIIB are both designated CD32, they are different proteins with distinct functions (see text). The neonatal FcR (FcRn) resembles class I major histocompatibility complex molecules structurally but does not have a peptide-binding cleft. *Ig,* Immunoglobulin; *ITAM,* immunoreceptor tyrosine-based activation motif;

ITIM, immunoreceptor tyrosine-based inhibition motif.

Role of Fcγ Receptors in Phagocytosis and Activation of Phagocytes

Binding of Fc receptors on phagocytes to particles coated with antibody molecules leads to engulfment of the particles and activation of the phagocytes (Fig. 13.4) **.** The IgG subtypes that bind best to Fcγ receptors (IgG1 and IgG3) are the most efficient opsonins for promoting phagocytosis. As discussed earlier, FcγRI is the high-affinity Fcγ receptor on phagocytic cells, and it is the most important receptor for phagocytosis of opsonized particles.

Opsonized particles are internalized into vesicles known as phagosomes, which fuse with lysosomes, and the phagocytosed particles are destroyed in these phagolysosomes. Activation requires cross-linking of the FcRs by several adjacent Ig molecules (e.g., on antibody-coated microbes or in immune complexes). Cross-linking of the ligand-binding α chains of an FcR results in signal transduction events that are similar to those that occur after antigen receptor cross-linking in lymphocytes (see Chapter 7). These include SRC kinase–mediated tyrosine phosphorylation of the ITAMs in the signaling chains of the FcRs; SH2 domain–mediated recruitment of SYK family kinases to the ITAMs; activation of phosphatidylinositol 3-kinase; recruitment of adaptor molecules, including SLP76 and BLNK; and recruitment of enzymes, such as phospholipase Cγ and TEC family kinases. These events lead to generation of inositol trisphosphate and diacylglycerol and sustained increase in cytosolic calcium.

The signaling pathways downstream of Fcγ receptors induce a number of responses in leukocytes, including transcription of genes encoding cytokines, inflammatory mediators, and microbicidal enzymes; and mobilization of the cytoskeleton, leading to phagocytosis, granule exocytosis, and cell migration. The major microbicidal substances produced in the activated phagocytes are reactive oxygen species, nitric oxide, and hydrolytic enzymes. These are the same substances produced by phagocytes activated in innate immune responses, discussed in Chapter 4. These microbicidal substances may also damage tissues; this mechanism of antibody-mediated tissue injury is important in hypersensitivity diseases (see Chapter 19). Knockout mice lacking the ligand-binding α chain of FcγRI or the signal-transducing FcR γ chain are defective in antibody-mediated defense against microbes and do not develop some forms of IgG antibody–mediated tissue injury, thus demonstrating the essential role of Fc receptors in these processes.

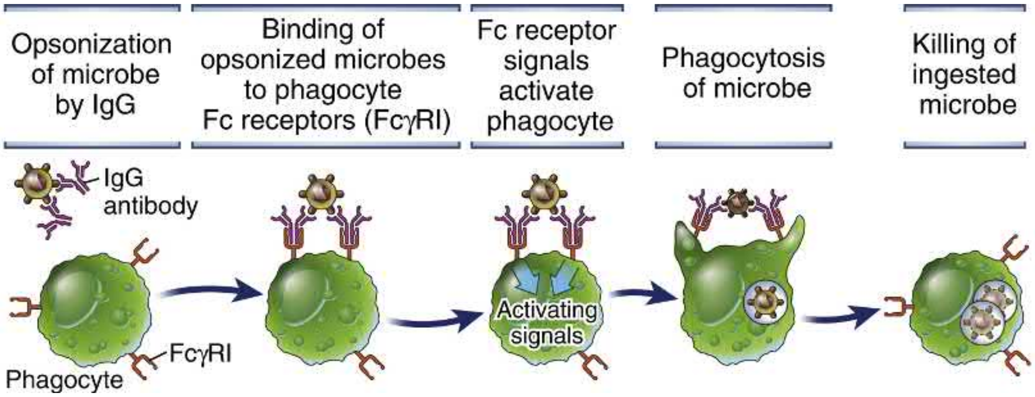

Opsonization of microbe by IgG
Binding of opsonized microbes to phagocyte Fc receptors (FcγRI)
Fc receptor signals activate phagocyte
Phagocytosis of microbe
Killing of ingested microbe
IgG antibody
Phagocyte
FcγRI
Activating signals

FIGURE 13.4 Antibody-mediated opsonization and phagocytosis of microbes.Antibodies of certain immunoglobulin G *(IgG)* subclasses bind to microbes and are then recognized by Fc receptors on phagocytes. Signals from the Fc receptors promote the phagocytosis of the opsonized microbes and activate the phagocytes to destroy these microbes. The microbicidal mechanisms of phagocytes are described in Chapters 4 (see Fig. 4.17) and 10 (see Fig. 10.7).

Inhibitory Signaling by the FcγRIIB Receptor

The FcγRIIB receptor is an inhibitory Fc receptor that was described earlier in the context of inhibitory signaling in B cells and the phenomenon of antibody feedback (see Chapter 12). FcγRIIB is also expressed on DCs, neutrophils, macrophages, and mast cells and may play a role in regulating the responses of these cells to activating Fc receptors and other stimuli. A somewhat empirical but often useful treatment of several autoimmune and inflammatory diseases is the intravenous administration of pooled human IgG, called intravenous immunoglobulin (IVIG). IVIG may bind to FcγRIIB and deliver inhibitory signals to B lymphocytes and myeloid cells, thus reducing antibody production and dampening inflammation. However, numerous other mechanisms have been proposed to explain how IVIG works.

Antibody-Dependent Cell-Mediated Cytotoxicity

NK cells and other leukocytes bind to antibody-coated cells by Fc receptors and destroy these cells. This process is called antibody-dependent cell-mediated cytotoxicity (ADCC) (Fig. 13.5). It was first described as a function of NK cells, which use their Fc receptor, FcγRIIIA (CD16), to bind to antibody-coated cells. FcγRIIIA is a low-affinity receptor that binds clustered IgG molecules displayed on cell surfaces but does not bind circulating monomeric IgG. Therefore, ADCC occurs only when the target cell is coated with antibody molecules, and free IgG in plasma neither activates NK cells nor competes effectively with cell-bound IgG for binding to FcγRIII. Engagement of FcγRIII by antibody-coated target cells activates the NK cells to synthesize and secrete cytokines, such as IFN-γ, as well as to discharge the contents of their granules, which mediate the killing functions of this cell type (see Chapter 4). ADCC also can be mediated by macrophages.

ADCC can be readily demonstrated in vitro, but its role in host defense against microbes is not established. It may be a mechanism for the elimination of cells that are coated by certain therapeutic monoclonal antibodies, such as B cells and B cell–derived tumor cells that are targeted by anti-CD20 antibody.

Antibody-Mediated Clearance of Helminths

Antibodies, eosinophils, and mast cells function together to mediate the killing and expulsion of some helminthic parasites. Helminths (worms) are too large to be engulfed by phagocytes, and their integuments are relatively resistant to the microbicidal products of neutrophils and macrophages. They can, however, be killed by a toxic cationic protein, known as the major basic protein, present in the granules of eosinophils. The immune response to helminthic parasites is dominated by Th2 cell activation, IgE antibody production, and eosinophila, suggesting that all may cooperate in defense. Antibodies that coat helminths, especially IgG, can bind to Fc receptors on eosinophils and cause the degranulation of the eosinophils, releasing cationic granule proteins and other mediators that kill the parasites. Neither human nor mouse eosinophils express appreciable levels of high affinity IgE receptors, and in human eosinophils this receptor lacks the signaling β chain, so a direct role of IgE in activating these cells seems unlikely. Human eosinophils can express the low-affinity IgE receptor (CD23), but the function of CD23 on these cells remains poorly understood. IgE antibodies that recognize antigens on the surface of the helminths may also initiate local mast cell or basophil degranulation through the high-affinity IgE receptor (see Chapter 20). Mast cell mediators can induce bronchoconstriction and increased intestinal motility, contributing to the expulsion of worms from sites such as the airways and the lumen of the gastrointestinal tract.

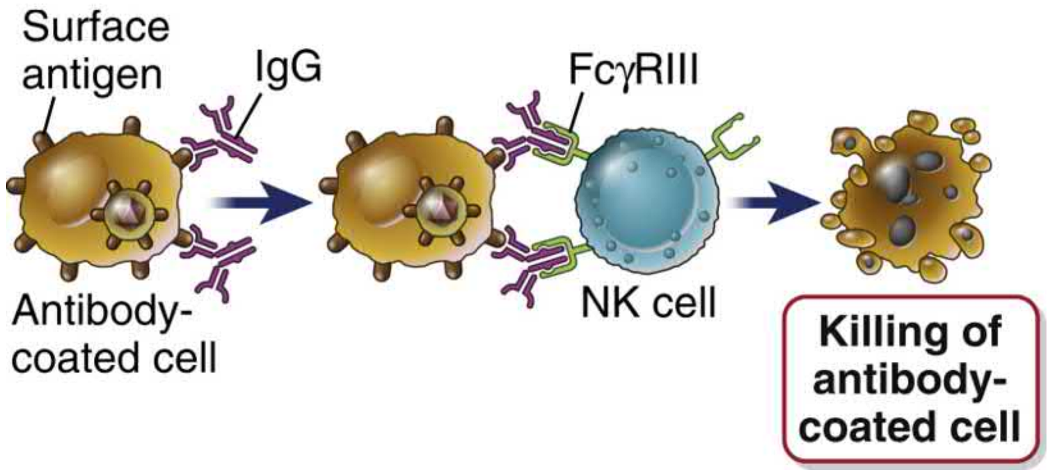


FIGURE 13.5 Antibody-dependent cell-mediated cytotoxicity.Antibodies of certain immunoglobulin G *(IgG)* subclasses bind to cells (e.g., infected cells), and the Fc regions of the bound antibodies are recognized by an Fcγ receptor on natural killer *(NK)* cells. The NK cells are activated and kill the antibody-coated cells.

The Complement System

The complement system is one of the major effector mechanisms of humoral immunity and is also an important effector mechanism of innate immunity. We briefly discussed the role of complement in innate immunity in Chapter 4. Here we will describe the activation and regulation of complement in more detail.

The name *complement* is derived from experiments performed by Jules Bordet shortly after the discovery of antibodies. He demonstrated that if fresh serum containing an antibacterial antibody is added to the bacteria at physiologic temperature (37°C), the bacteria are lysed. If, however, the serum is heated to 56°C or more, it loses its lytic capacity. This loss of lytic capacity is not due to decay of antibody activity, because antibodies are relatively heat stable, and even heated serum is capable of agglutinating the bacteria. The lytic capacity of the serum could be restored by adding fresh serum, even from animals that had not been immunized. Bordet concluded that in order to lyse bacteria, serum must contain another heat-labile component that is present in all individuals and assists, or complements, the lytic function of antibodies. This component was later given the name **complement.**

The complement system consists of serum and cell surface proteins that interact with one another and with other molecules of the immune system in a highly regulated manner to generate products that function to eliminate microbes. Complement proteins are plasma proteins that are normally inactive; they are activated only under particular conditions to generate products that mediate various effector functions. Several features of complement activation are essential for its normal function.

- ***The complement system is activated by microbes and by antibodies and lectins that are bound to microbes and other antigens.*** Thus, complement focuses immune attack on microbial surfaces. The mechanisms of initial activation are described later.
- ***Activation of complement involves the sequential proteolysis of proteins to generate enzyme complexes with proteolytic activity.*** Proteins that acquire proteolytic enzymatic activity by the action of other proteases are called zymogens. The process of sequential zymogen activation, a defining feature of a proteolytic enzyme cascade, is also characteristic of the coagulation and kinin systems. Proteolytic cascades allow tremendous and rapid amplification because each active enzyme molecule produced at one step can generate multiple activated enzyme molecules at the next step.
- ***Several of the biologically active cleavage products of complement activation become covalently attached to microbial cell surfaces, to antibodies bound to microbes and other antigens, and to apoptotic bodies.*** In the fluid phase, complement proteins are inactive or only transiently active (for seconds), but they become stably activated after they are attached to microbes, antibodies, or dying cells. Thus, the full activation and therefore the biologic functions of

the complement system are limited to microbial cell surfaces or to sites of antibodies bound to antigens and do not occur in the blood.

- ***Complement activation is inhibited by regulatory proteins that are present on normal host cells and absent from microbes.*** The regulatory proteins are an adaptation of normal cells that minimize complement-mediated damage to host cells. Because microbes lack these regulatory proteins, complement activation can occur on microbial surfaces.

Pathways of Complement Activation

There are three pathways of complement activation: the classical pathway, which is activated by IgM and IgG antibodies bound to antigens; the alternative pathway, which is activated on microbial cell surfaces in the absence of antibody; and the lectin pathway, which is activated by plasma lectins that bind to surface carbohydrates on microbes (Fig. 13.6) **.** The names classical and alternative arose because the classical pathway was discovered and characterized first, but the alternative pathway is phylogenetically older. The alternative and lectin pathways are effector mechanisms of innate immunity, whereas the classical pathway is a major mechanism of adaptive humoral immunity.

The central event in complement activation is proteolysis of the complement protein C3 to generate biologically active products and the subsequent covalent attachment of a product of C3, called C3b, to microbial cell surfaces or to antibody bound to antigen (see Fig. 13.6) **.** Although the pathways of complement activation differ in how they are initiated, all of them result in cleavage of the most abundant complement protein, C3. Complement activation involves the generation of a proteolytic complex, the **C3 convertase,** which cleaves C3 into two fragments called C3a and C3b. (By convention, the proteolytic products of each complement protein are identified by lowercase letter suffixes, *a* referring to the smaller product and *b* to the larger one; C2 is an exception, for historical reasons.) C3b becomes covalently attached to the microbial cell surface or to antibody molecules bound to antigen. All of the biologic functions of complement are dependent on the proteolytic cleavage of C3. For example, complement activation promotes phagocytosis because C3b becomes covalently linked to microbes, and phagocytes (neutrophils and macrophages) express receptors for C3b. Peptides produced by proteolysis of C3 (and other complement proteins) stimulate inflammation.

In all three pathways of complement activation, after the generation of C3b by the C3 convertase, a second enzyme complex called the **C5 convertase** is assembled, which cleaves C5 into C5a and C5b. The C5 convertase contributes both to inflammation by generation of the C5a fragment, and to the formation of pores in the membranes of microbial targets. The pathways of complement activation differ in how C3b is produced but follow a shared sequence of reactions after the cleavage of C5.

With this background, we proceed to more detailed descriptions of the alternative, classical, and lectin pathways.

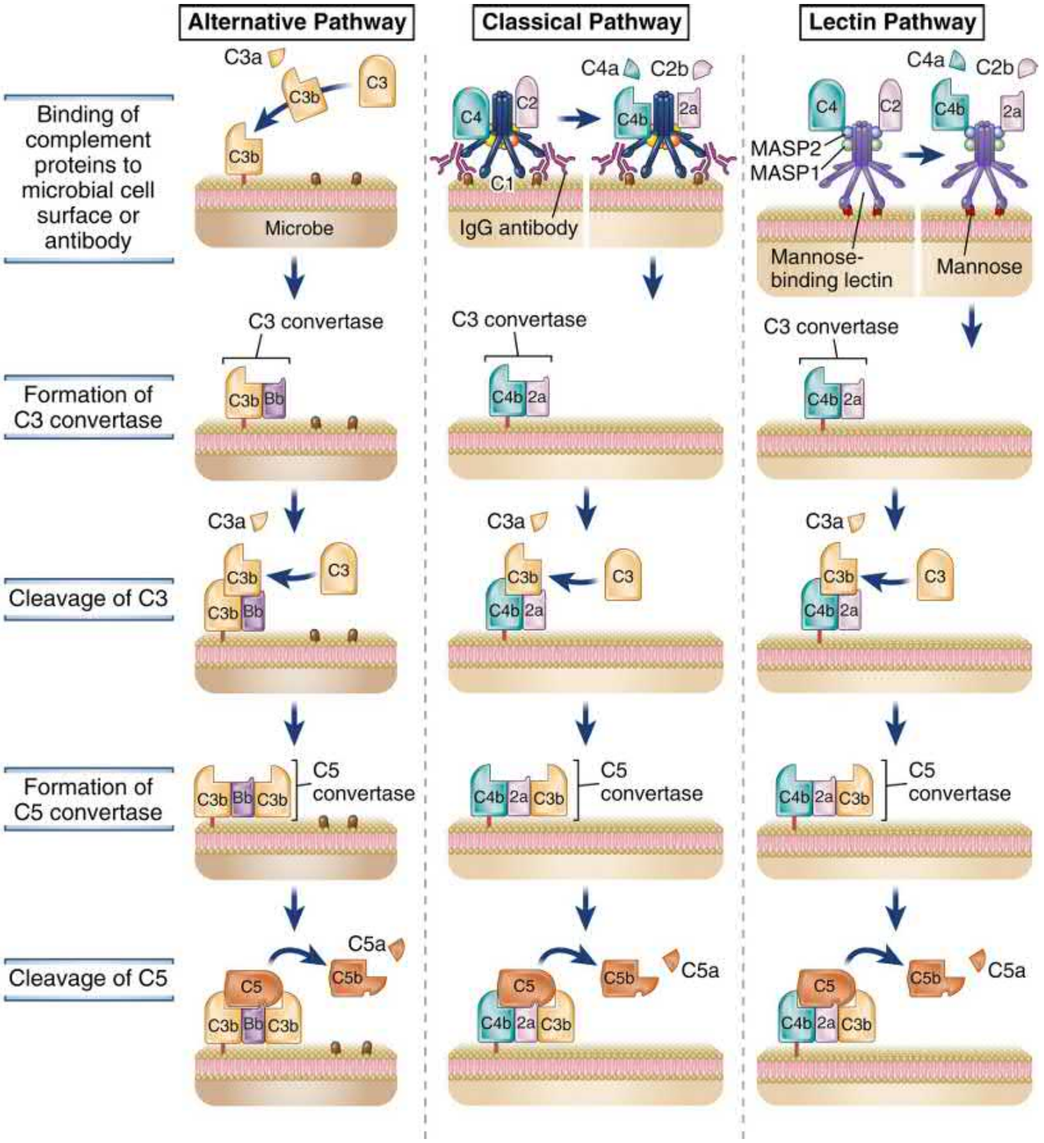

Alternative Pathway
Classical Pathway
Lectin Pathway
Binding of complement proteins to microbial cell surface or antibody
C3a
C3b
C3
C3b
Microbe
C4a
C2b
C4
C2
C4b
2a
C1
IgG antibody
C4a
C2b
C4
C2
C4b
2a
MASP2
MASP1
Mannose-binding lectin
Mannose
Formation of C3 convertase
C3 convertase
C3b
Bb
C3 convertase
C4b
2a
C3 convertase
C4b
2a
Cleavage of C3
C3a
C3b
C3
C3b
Bb
C3a
C3b
C3
C4b
2a
C3a
C3b
C3
C4b
2a
Formation of C5 convertase
C3b
Bb
C3b
C5 convertase
C4b
2a
C3b
C5 convertase
C4b
2a
C3b
C5 convertase
Cleavage of C5
C5a
C5
C5b
C3b
Bb
C3b
C5
C5b
C5a
C4b
2a
C3b
C5
C5b
C5a
C4b
2a
C3b

FIGURE 13.6 The early steps of complement activation by the alternative, classical, and lectin pathways.The alternative pathway is activated by C3b binding to various activating surfaces, such as microbial cell walls; the classical pathway is initiated by C1 binding to antigen-antibody complexes, and the lectin pathway is activated by binding of a plasma lectin to microbes. The C3b that is generated by the action of the C3 convertase binds to the microbial cell surface or the antibody and becomes a component of the

enzyme that cleaves C5 (C5 convertase) and initiates the late steps of complement activation. The late steps of all three pathways are the same (not shown), and complement activated by all three pathways serves the same functions. *MASP,* Mannose-binding lectin-associated serine protease.

The Alternative Pathway

The alternative pathway of complement activation results in the proteolysis of C3 and the stable attachment of its breakdown product C3b to microbial surfaces, without a role for antibody (Fig. 13.7 and Table 13.4) **.** Normally, C3 in plasma is being continuously hydrolyzed and then cleaved at a low rate (1% to 2% of the total plasma C3 per hour) to generate C3b in a process that is called C3 tickover. This process involves Factors B and D, described later. The C3 protein contains a reactive thioester bond that is buried in a region of the protein known as the thioester domain. When C3 is cleaved, the C3b molecule undergoes a dramatic conformational change and the thioester domain flips out (a large shift of approximately 85 Å), exposing the previously hidden reactive thioester bond. A small amount of the C3b may become covalently attached to the surfaces of cells, including microbes, through the thioester domain, which reacts with the amino or hydroxyl groups of cell surface proteins or polysaccharides to form amide or ester bonds (Fig. 13.8). If these bonds are not formed, the C3b remains in the fluid phase, and the exposed reactive thioester bond is quickly hydrolyzed, rendering the protein inactive. As a result, further complement activation cannot proceed in plasma.

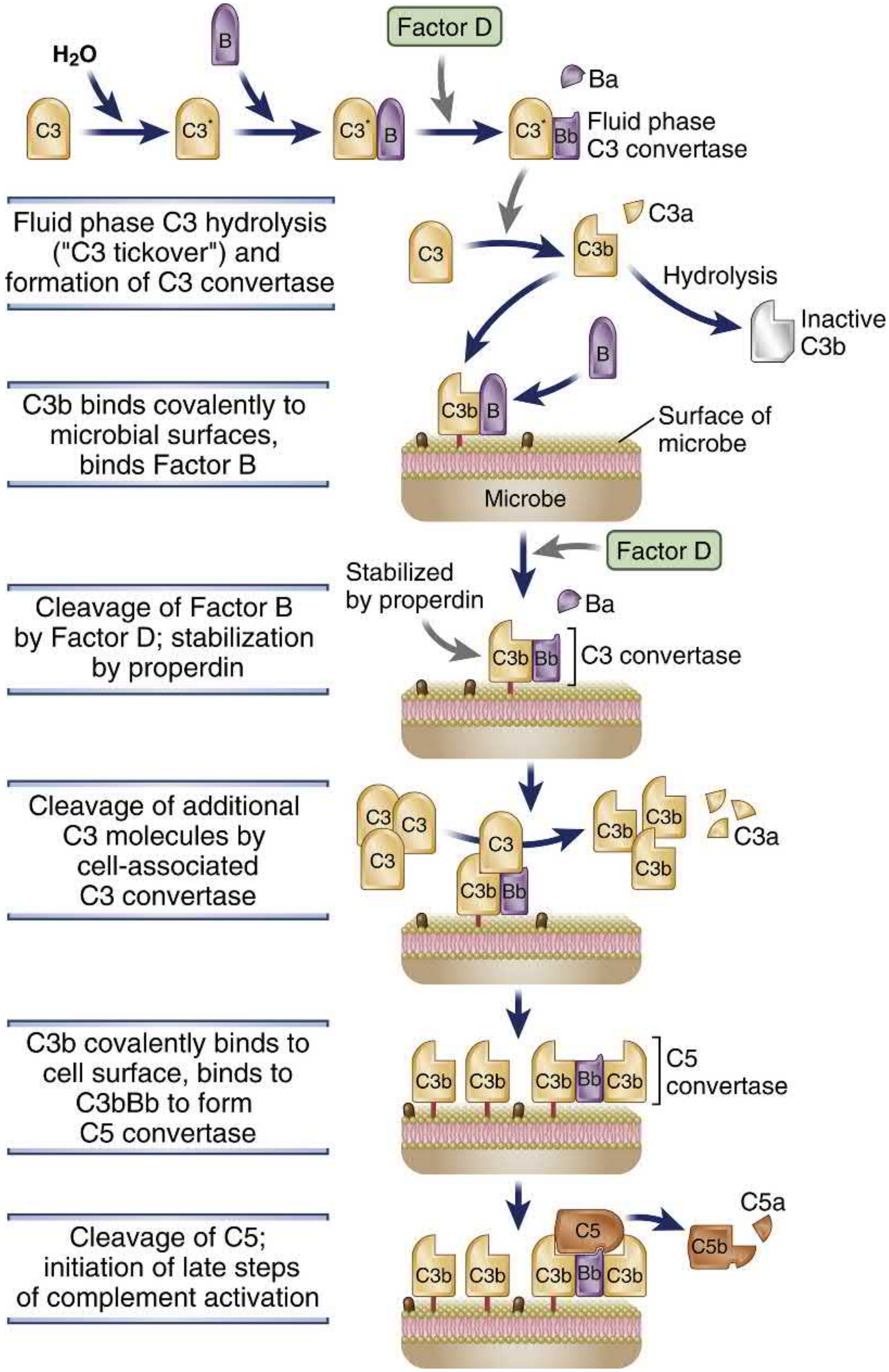

H2O
B
Factor D
Ba
C3
C3*
C3* B
C3* Bb
Fluid phase C3 convertase
Fluid phase C3 hydrolysis ("C3 tickover") and formation of C3 convertase
C3
C3b
C3a
Hydrolysis
Inactive C3b
C3b binds covalently to microbial surfaces, binds Factor B
B
C3b B
Surface of microbe
Microbe
Factor D
Stabilized by properdin
Ba
C3b Bb
C3 convertase
Cleavage of Factor B by Factor D; stabilization by properdin
Cleavage of additional C3 molecules by cell-associated C3 convertase
C3
C3
C3
C3
C3b Bb
C3b
C3b
C3b
C3a
C3b covalently binds to cell surface, binds to C3bBb to form C5 convertase
C3b
C3b
C3b Bb C3b
C5 convertase
Cleavage of C5; initiation of late steps of complement activation
C5
C5a
C5b
C3b
C3b
C3b Bb C3b

FIGURE 13.7 The alternative pathway of complement activation.Spontaneous hydrolysis of

plasma C3 leads to the formation of a fluid-phase C3 convertase and the generation of C3b in the process called C3 tickover. The activated hydrolyzed C3, indicated as $C3^{*}$, is deposited on microbial surfaces, where it binds Factor B and forms the alternative pathway C3 convertase. This convertase (C3bBb) cleaves C3 to produce more C3b, which binds to the microbial surface and participates in the formation of a C5 convertase. The C5 convertase, C3bBbC3b, cleaves C5 to generate C5b, the initiating event in the late steps of complement activation.

TABLE 13.4

Protein	Structure	Serum Concentration (μg/mL)	Function
C3	185 kD (α subunit, 110 kD; β subunit, 75 kD)	1400–1700	C3b binds to the surface of the microbe, where it functions as an opsonin and as a component of C3 and C5 convertases.
			C3a stimulates inflammation (anaphylatoxin).
Factor B	93-kD monomer	200–400	Bb is a serine protease and the active enzyme of the C3 and C5 convertases.
Factor D	25-kD monomer	1–3	Plasma serine protease cleaves factor B when it is bound to C3b.
Properdin	Composed of up to four 56-kD subunits	20–35	Properdin stabilizes C3 convertases (C3bBb) on microbial surfaces.

When C3b undergoes its post-cleavage conformational change, a binding site for a plasma protein called Factor B is also exposed. Factor B then binds to the C3b protein that is now covalently tethered to the surface of the cell. Bound Factor B is in turn cleaved by a plasma serine protease called Factor D, releasing a small fragment called Ba and generating a larger fragment called Bb that remains attached to C3b. The C3bBb complex is the alternative pathway C3 convertase and functions to cleave more C3 molecules, thus setting up an amplification sequence. Even when C3b is generated by the classical or lectin pathway, it can form a complex with Bb, and this complex is able to cleave more C3. Thus, the alternative pathway C3 convertase functions to amplify complement activation when it is initiated by any of the three pathways. When C3 is broken down, C3b remains attached to cells and C3a is released. The soluble fragment has several biologic activities that are discussed later.

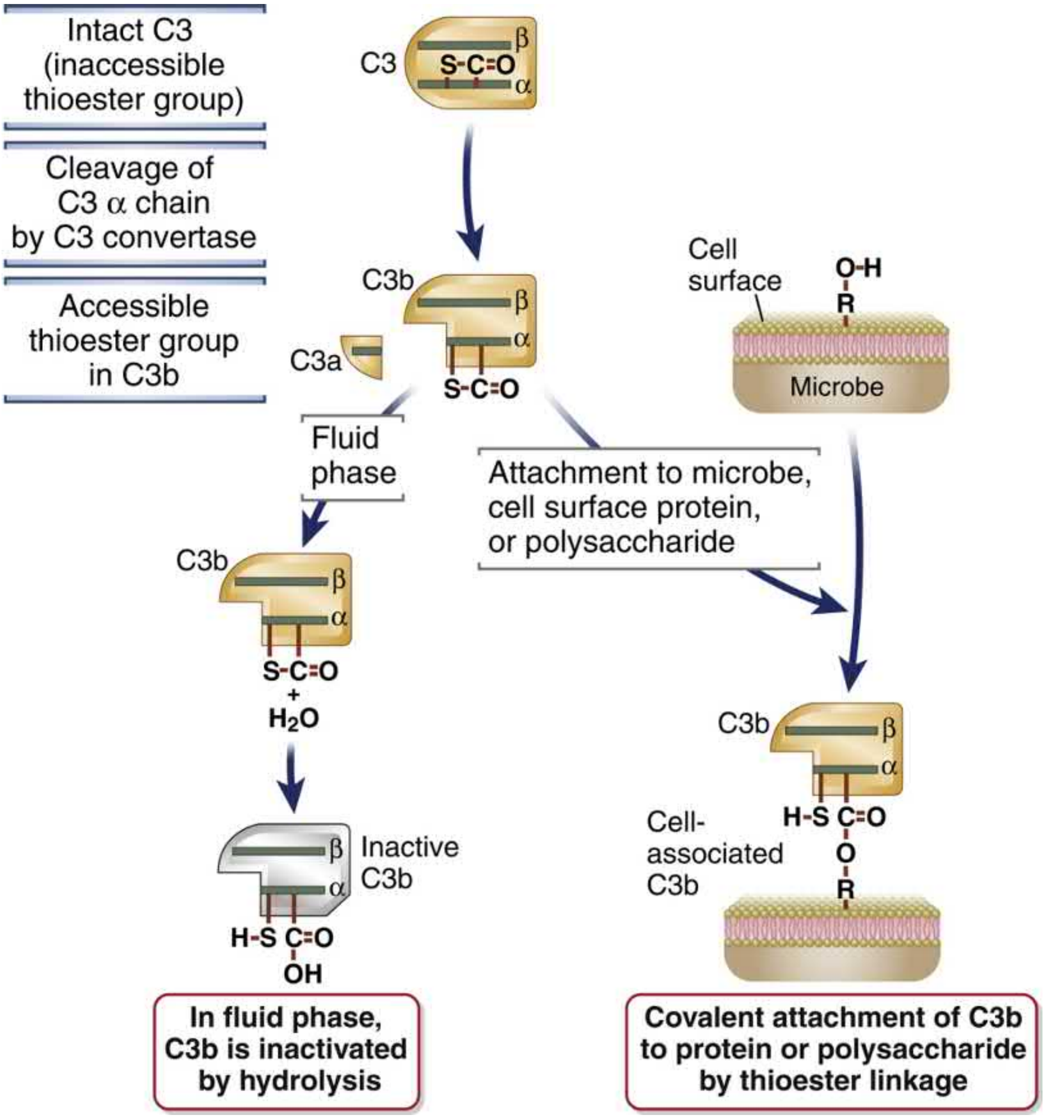

Intact C3 (inaccessible thioester group)
Cleavage of C3 α chain by C3 convertase
Accessible thioester group in C3b
C3
S-C=O
β
α
C3b
C3a
Cell surface
O-H
R
Microbe
Fluid phase
Attachment to microbe, cell surface protein, or polysaccharide
S-C=O
+
H2O
Inactive C3b
H-S C=O
OH
Cell-associated C3b
H-S C=O
O
R
In fluid phase, C3b is inactivated by hydrolysis
Covalent attachment of C3b to protein or polysaccharide by thioester linkage

FIGURE 13.8 Internal thioester bonds of C3.Proteolytic cleavage of the α chain of C3 converts it into a metastable form in which the internal thioester bonds are exposed and susceptible to nucleophilic attack by oxygen atoms (as shown) or nitrogen atoms. The result is the formation of covalent bonds with proteins or carbohydrates on cell surfaces. C4 is structurally homologous to C3 and has an identical thioester bond.

Alternative pathway activation readily occurs on microbial cell surfaces but not on mammalian cells. If the C3bBb complex is formed on mammalian cells, it is rapidly degraded and the reaction is terminated by the action of several regulatory proteins present on these cells (discussed later). Lack of the regulatory proteins on microbial cells allows binding and activation of the alternative pathway C3 convertase. In addition, another protein of the alternative pathway, called properdin, can bind to and stabilize the C3bBb complex, and the attachment of properdin is favored on microbial as opposed to normal host cells.

Some of the C3b molecules generated by the alternative pathway C3 convertase bind to the convertase itself. This results in the formation of a complex containing one Bb moiety and two molecules of C3b, which functions as the alternative pathway C5 convertase, which will cleave C5 and initiate the late steps of complement activation.

The Classical Pathway

The classical pathway is initiated by binding of the complement protein C1 to the C_H2 domain of IgG or the C_H3 domain of IgM molecules that have bound antigen (Fig. 13.9 and Table 13.5) **.** Among IgG antibodies, IgG1 and IgG3 (in humans) are more efficient activators of complement than are other subclasses. IgG2 has some ability to activate complement, but IgG4 does not. C1 is a large, multimeric protein complex composed of C1q, C1r, and C1s subunits; C1q binds to the antibody, and C1r and C1s are proteases. The C1q subunit is made up of an umbrella-like radial array of six chains, each of which has a globular head connected by a collagen-like arm to a central stalk (Fig. 13.10). This hexamer performs the recognition function of the molecule and binds specifically to the Fc regions of μ and some γ heavy chains. As mentioned in Chapter 4, C1q binds to pentraxins such as C-reactive protein and serum amyloid protein and also can bind to apoptotic bodies.

Only antibodies bound to antigens, and not free circulating antibodies, can initiate classical pathway activation. The reason for this is that each C1q molecule must bind to at least two Ig heavy chains to be activated and each Ig Fc region has only a single C1q-binding site. Therefore, two or more Fc regions have to be accessible to C1 in order to initiate classical pathway activation. Because each IgG molecule has only one Fc region, multiple IgG molecules must be brought close together before C1q can bind, and multiple IgG antibodies are brought together only when they simultaneously bind to identical epitopes of a multivalent antigen or to several antigen molecules on a microbe, cell, or tissue surface (Fig. 13.11). Even though free (circulating) IgM is pentameric, it does not bind C1q because the C_H3 domains of free IgM are in a configuration that is inaccessible to C1q. Binding of the IgM to an antigen induces a conformational change that exposes the C1q binding sites in the C_H3 domains and allows C1q to bind. Because of its pentameric structure, a single molecule of IgM can bind multiple C1q molecules, and this is one reason that IgM is a more efficient complement-binding (also called complement-fixing) antibody than is IgG.

C1r and C1s are serine proteases that form a tetramer containing two molecules of each protein. Binding of two or more of the globular heads of C1q to the Fc regions of IgG or IgM leads to enzymatic activation of the associated C1r, which cleaves and activates C1s (see Fig. 13.9). Activated C1s cleaves the next protein in the cascade, C4, to generate C4b. (The smaller C4a fragment is released and has biologic activities that are described later.) C4 is homologous to C3, and C4b contains an internal thioester bond, similar to that in C3b, that forms covalent amide or ester linkages with the antigen-antibody complex or with the adjacent surface of a cell to which the antibody is bound. This attachment of C4b ensures that classical pathway activation proceeds on a cell surface or immune complex. The next complement protein, C2, then complexes with the cell surface–bound C4b and is cleaved by a nearby C1s molecule to generate a soluble C2b fragment of unknown importance and a larger C2a fragment that remains physically associated with C4b on the cell surface. The resulting

C4b2a complex is the classical pathway C3 convertase; it has the ability to bind to and proteolytically cleave C3. Binding of this enzyme complex to C3 is mediated by the C4b component, and proteolysis is catalyzed by the C2a component. Cleavage of C3 results in removal of the small C3a fragment, and C3b can form covalent bonds with cell surfaces or with the antibody where complement activation was initiated. After C3b is deposited, it can bind Factor B and generate more C3 convertase by the alternative pathway, as discussed earlier. The net effect of the multiple enzymatic steps and amplification is that millions of molecules of C3b can be deposited within minutes on the cell surface where complement is activated. The key early steps of the alternative and classical pathways are analogous: C3 in the alternative pathway is homologous to C4 in the classical pathway, and Factor B is homologous to C2.

Some of the C3b molecules generated by the classical pathway C3 convertase bind to the convertase (as in the alternative pathway) and form a C4b2a3b complex. This complex functions as the classical pathway C5 convertase; it cleaves C5 and initiates the late steps of complement activation.

The Lectin Pathway

The lectin pathway of complement activation is triggered by the binding of microbial polysaccharides to circulating lectins, such as plasma mannose-binding lectin (MBL), or to ficolins (Table 13.6) **.** These soluble lectins are collagen-like proteins that structurally resemble C1q (see Fig. 4.13). MBL, L-ficolin, and H-ficolin are plasma proteins; M-ficolin is mainly secreted by activated macrophages in tissues. MBL has an N-terminal collagen-like domain and a C-terminal carbohydrate recognition (lectin) domain and is thus a member of the collectin family of serum agglutinins. The ficolins have a similar structure, with an N-terminal collagen-like domain and a C-terminal fibrinogen-like domain. The collagen-like domains help to assemble basic triple-helical structures that can form higher order oligomers. MBL binds to mannose residues on polysaccharides, and the fibrinogen-like domain of ficolin binds N-acetylglucosamine–containing glycans. These polysaccharides and glycans are abundant in bacteria and fungi. Both MBL and ficolins associate with MBL-associated serine proteases (MASPs) including MASP1, MASP2, and MASP3 (see Table 13.6). The MASPs are structurally homologous to the C1r and C1s proteases and serve a similar function, namely the cleavage of C4 and C2 to activate the complement pathway. Multimers of MBL associate with MASP1 and MASP2 (or MASP3 and MASP2), and MASP2 is the protease that cleaves C4 and C2. Subsequent events in this pathway are identical to those that occur in the classical pathway.

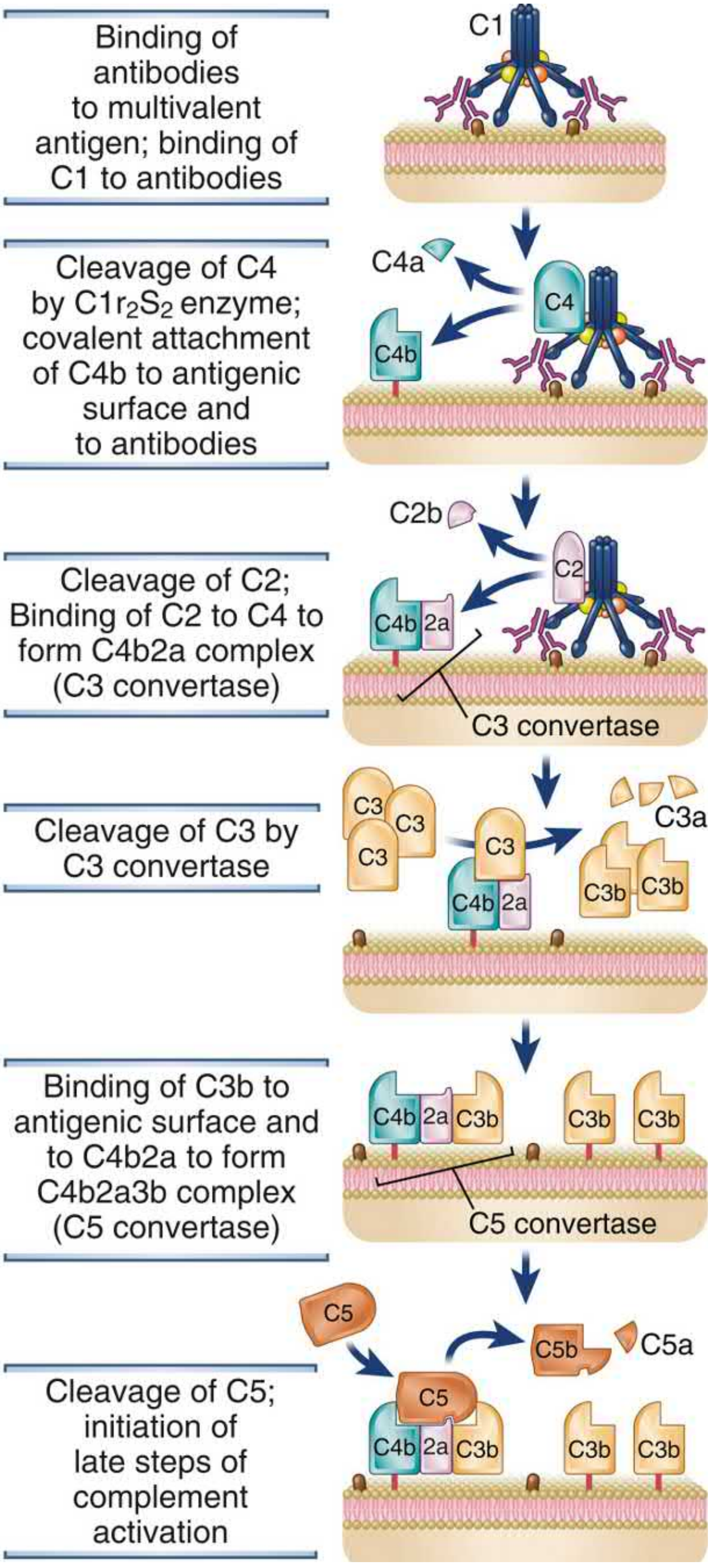

Binding of antibodies to multivalent antigen; binding of C1 to antibodies
C1
Cleavage of C4 by C1r$_2$S$_2$ enzyme; covalent attachment of C4b to antigenic surface and to antibodies
C4a
C4
C4b
C2b
Cleavage of C2; Binding of C2 to C4 to form C4b2a complex (C3 convertase)
C2
C4b
2a
C3 convertase
Cleavage of C3 by C3 convertase
C3
C3
C3
C3
C4b
2a
C3a
C3b
C3b
Binding of C3b to antigenic surface and to C4b2a to form C4b2a3b complex (C5 convertase)
C4b
2a
C3b
C3b
C3b
C5 convertase
C5
C5b
C5a
Cleavage of C5; initiation of late steps of complement activation
C5
C4b
2a
C3b
C3b
C3b

FIGURE 13.9 The classical pathway of complement activation.Antigen-antibody complexes that activate the classical pathway may be soluble, fixed on the surface of cells (as shown), or deposited on extracellular matrices. The classical pathway is initiated by the binding of C1 to antigen-complexed antibody molecules, which leads to the production of C3 and C5 convertases attached to the surfaces where the antibody was deposited. The C5 convertase cleaves C5 to begin the late steps of complement activation.

TABLE 13.5

Protein	Structure	Serum Concentration (μg/mL)	Function
C1 (C1qr2s2)	750 kD	—	Initiates the classical pathway
C1q	460 kD; hexamer of three pairs of chains (22, 23, 24 kD)	50–150	Binds to the Fc portion of antibody that has bound antigen, to apoptotic cells, and to cationic surfaces
C1r	85-kD dimer	50	Serine protease, cleaves C1s to make it an active protease
C1s	85-kD dimer	50	Serine protease, cleaves C4 and C2
C4	210 kD, trimer of 97-, 75-, and 33-kD chains	300–600	C4b covalently binds to the surface of a microbe or cell, where antibody is bound and complement is activated.
			C4b binds C2 for cleavage by C1s.
			C4a stimulates inflammation (anaphylatoxin).
C2	102-kD monomer	20	C2a is a serine protease and functions as the active enzyme of C3 and C5 convertases to cleave C3 and C5.
C3	See Table 13.4		

Late Steps of Complement Activation

C5 convertases generated by the alternative, classical, or lectin pathway initiate activation of the late components of the complement system, which culminates in formation of the cytocidal membrane attack complex (MAC) (Table 13.7 and Fig. 13.12) **.** C5 convertases cleave C5 into a small C5a fragment that is released and a two-chain C5b fragment (containing an α and a β chain) that is also released but binds rapidly to plasma C6. C6 undergoes a conformational change, and the C5b-C6 complex then binds to the cell membrane through both ionic and hydrophobic interactions. C5a has potent biologic effects on several cells that are discussed later. C7 from the plasma then binds to the α chain of C5b and forms the C5b-C6-C7 (C5b-7) complex. The bound C7 undergoes an amphiphilic transition and penetrates the membrane; it can contribute to the release of some phospholipid micelles from the membrane but does not form complete pores. The C8 protein is a trimer composed of three distinct chains, one of which binds to the C5b component of the C5b-7 complex and forms a covalent heterodimer with the second chain; the third chain inserts into the lipid bilayer of the membrane. This stably inserted C5b,6,7,8 complex (C5b-8) forms unstable pores that range from 0.4 to 3 nm in diameter, and very large numbers of these C5b-8 complexes can lyse cells. The formation of a fully active MAC is accomplished by the binding of C9, the final component of the complement cascades, to the C5b-8 complex. C9 is a serum protein that polymerizes at the site of the bound C5b-8 to form pores in plasma membranes that are made up of C5b-9 complexes containing C5b, C6, C7, C8, and many molecules of C9. These pores are approximately 20 nm in external diameter, 1 to 11 nm in internal diameter, have a height of approximately 15 nm, and form channels that allow free movement of water and ions. The channel size varies based on the number of C9 molecules in the C5b-C9 complex. Tubular complexes of C9 alone may also form. The entry of water results in osmotic swelling and rupture of the cells on whose surface the MAC is deposited. The pores formed by polymerized C9 are similar to the membrane pores formed by perforin, the cytolytic granule protein found in cytotoxic T lymphocytes and NK cells (see Chapter 11), and C9 is structurally homologous to perforin.

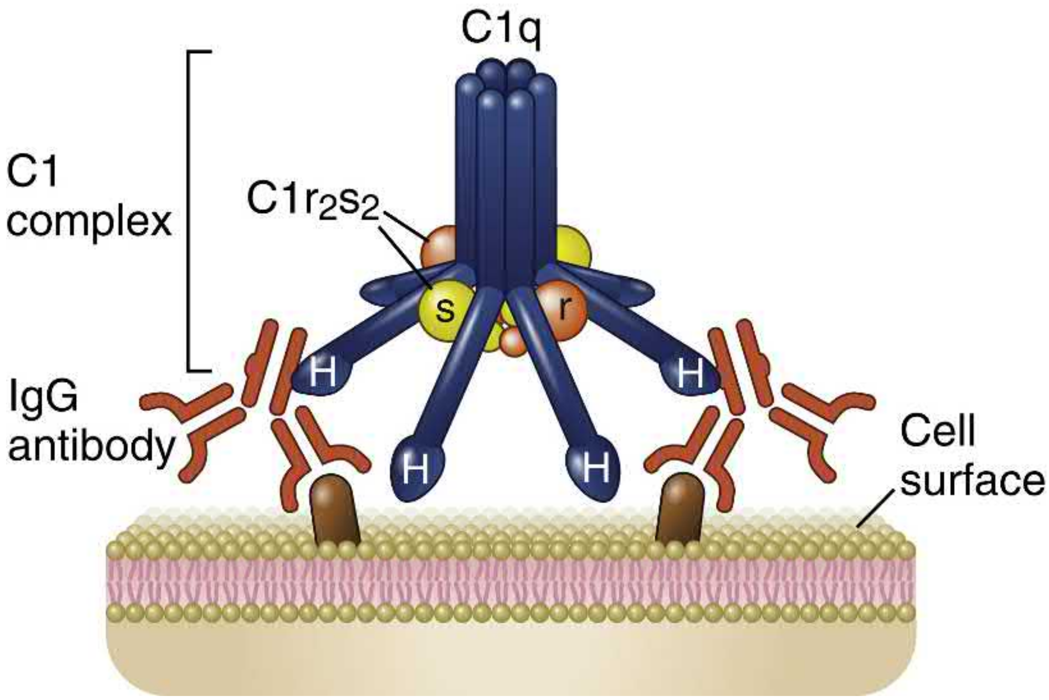


FIGURE 13.10 Structure of C1.C1q consists of six identical subunits arranged to form a central core and symmetrically projecting radial arms. The globular heads at the end of each arm, designated H, are the contact regions for the Fc portions of antibodies (IgG, in this case). C1r and C1s form a tetramer composed of two C1r and two C1s molecules. The ends of C1r and C1s contain the catalytic domains of these proteins. One C1r2s2 tetramer wraps around the radial arms of the C1q complex in a manner that juxtaposes the catalytic domains of C1r and C1s.

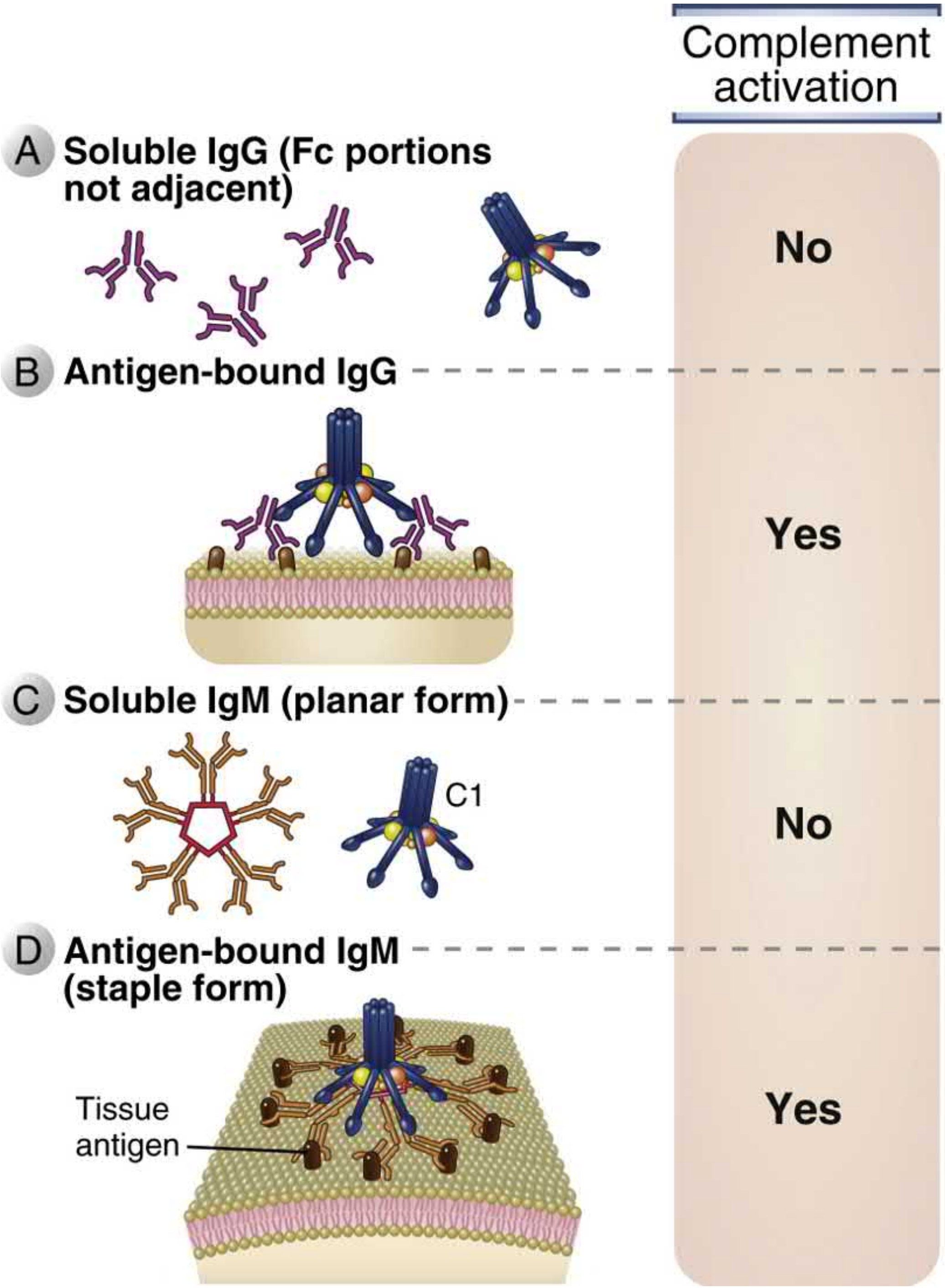

Complement activation
A Soluble IgG (Fc portions not adjacent)
No
B Antigen-bound IgG
Yes
C Soluble IgM (planar form)
C1
No
D Antigen-bound IgM (staple form)
Tissue antigen
Yes

FIGURE 13.11 C1 binding to the Fc portions of immunoglobulins M and G.C1 must bind to two or more Fc portions to initiate the complement cascade. Soluble IgG molecules will not activate C1 because each IgG has only one Fc region *(A)*, but after binding to cell surface antigens, adjacent

IgG Fc portions can bind and activate C1 *(B).* The Fc portions of soluble pentameric IgM are not accessible to C1 *(C).* After IgM binds to surface-bound antigens, it undergoes a shape change that permits C1 binding and activation *(D).*

TABLE 13.6

Protein	Structure	Serum Concentration (μg/mL)	Function
Mannose-binding lectin	Helical trimer of 32-kD chain; dimers to hexamers of this triple helix	1–8	Agglutinin, opsonin, complement fixing
M-ficolin (ficolin-1)	Helical trimer of 34-kD chain; a tetramer of this triple helix	Undetectable	Agglutinin, opsonin, complement fixing
L-ficolin (ficolin-2)	Helical trimer of 34-kD chain; a tetramer of this triple helix	1–7	Agglutinin, opsonin, complement fixing
H-ficolin (ficolin-3)	Helical trimer of 34-kD chain; a tetramer of this triple helix	6–83	Agglutinin, opsonin, complement fixing
MASP1	90-kD homodimer; homology to C1r/C1s	2–13	Forms complex with MASP2 and collectins or ficolins and activates MASP3
MASP2	110-kD homodimer; homology to C1r/C1s	2–13	Forms complex with lectins, especially ficolin-3
MASP3	76-kD homodimer; homology to C1r/C1s	0.02–1.0	Associates with collectins or ficolins and MASP1 and cleaves C4

The serum concentrations indicated are approximations for some of these proteins.

MASP, Mannose-binding receptor-associated serine protease.

TABLE 13.7

Protein	Structure	Serum Concentration (μg/mL)	Function
C5	190-kD dimer of 115- and 75-kD chains	80	C5b initiates assembly of the MAC
			C5a stimulates inflammation (anaphylatoxin)
C6	110-kD monomer	45	Component of the MAC: binds to C5b and accepts C7
C7	100-kD monomer	90	Component of the MAC: binds to C5b,6 and inserts into lipid membranes
C8	155-kD trimer of 64-, 64-, and 22-kD chains	60	Component of the MAC: binds to C5b,6,7 and initiates the binding and polymerization of C9
C9	79-kD monomer	60	Component of the MAC: binds to C5b,6,7,8 and polymerizes to form membrane pores

MAC, Membrane attack complex.

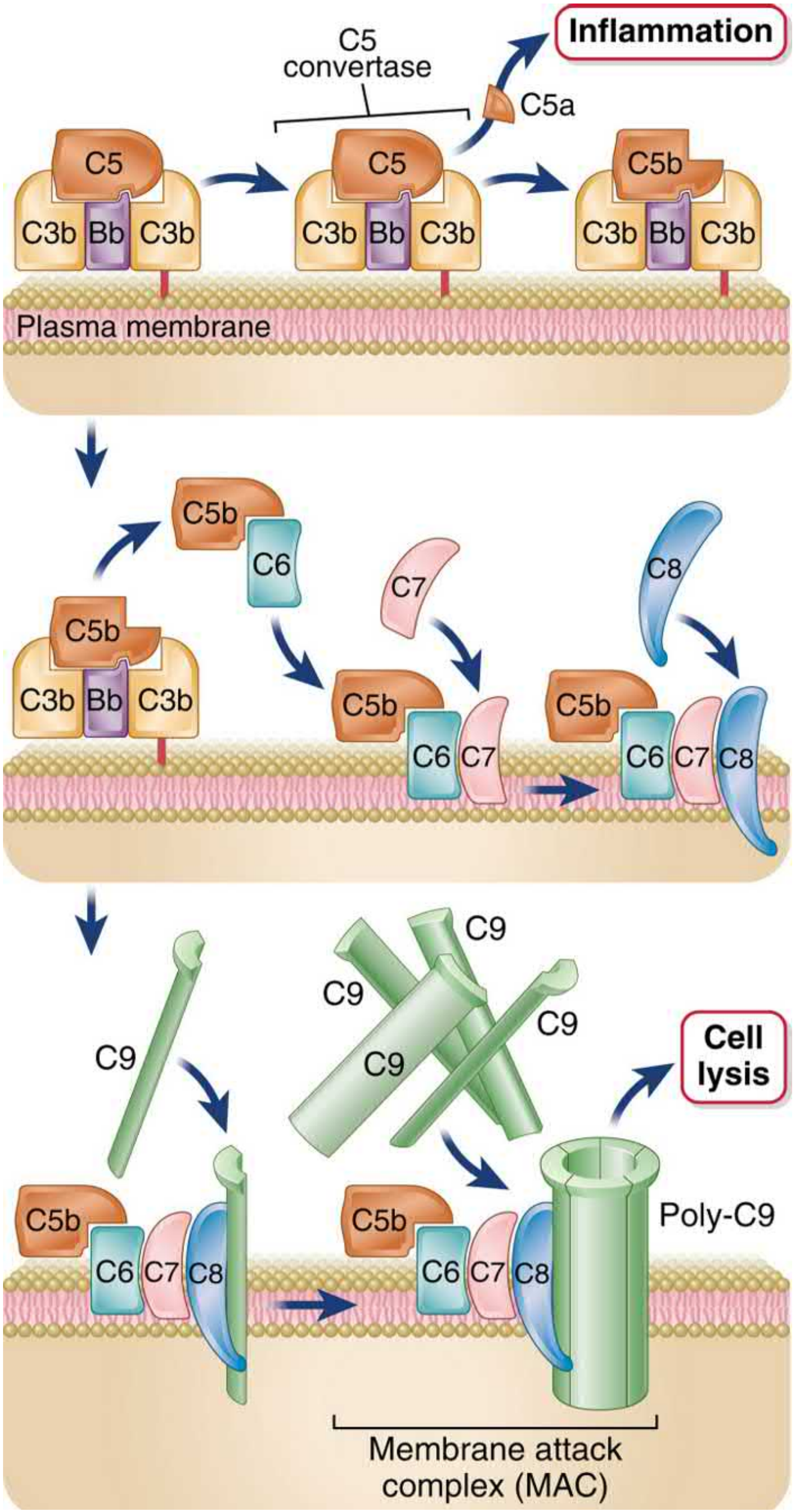

Inflammation
C5 convertase
C5a
C5
C3b
Bb
C3b
C5
C3b
Bb
C3b
C5b
C3b
Bb
C3b
Plasma membrane
C5b
C6
C7
C8
C5b
C3b
Bb
C3b
C5b
C6
C7
C5b
C6
C7
C8
C9
C9
C9
C9
C9
Cell lysis
C5b
C6
C7
C8
C5b
C6
C7
C8
Poly-C9
Membrane attack complex (MAC)

FIGURE 13.12 Late steps of complement activation and formation of the membrane attack complex.The cell-associated C5 convertase cleaves C5 and generates C5b, becomes bound to the convertase. C5b binds C6 and C7 sequentially, and the C5b-7 complex inserts into the plasma membrane, followed by addition of C8 to the complex, which forms unstable pores. The C5b-8 complex can form a pore with C9, and C9 can also be induced to homo-oligomerize by the C5b-8 complex. As many as 15 C9 molecules may polymerize to form the membrane attack complex (MAC), which creates pores in the membrane and induces cell lysis. C5a released on proteolysis of C5 stimulates inflammation.

Receptors for Complement Proteins

Many of the biologic activities of the complement system are mediated by the binding of complement fragments to membrane receptors expressed on various cell types. The best characterized of these receptors are specific for fragments of C3 and are described here (Table 13.8).

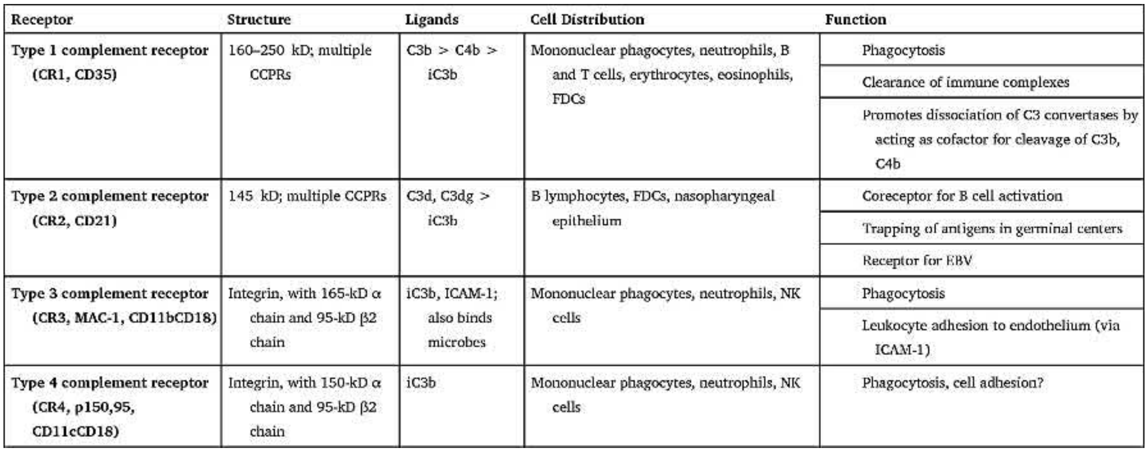
- ***The type 1 complement receptor (CR1, or CD35) functions mainly to promote phagocytosis of C3b- and C4b-coated particles and clearance of immune complexes from the circulation.*** CR1 is a high-affinity receptor for C3b and C4b. It is expressed mainly on bone marrow–derived cells, including erythrocytes, neutrophils, monocytes, macrophages, eosinophils, and T and B lymphocytes; it is also found on follicular dendritic cells (FDCs) in the follicles of secondary lymphoid organs. Phagocytes use this receptor to bind and internalize particles opsonized with

C3b or C4b. The binding of C3b- or C4b-coated particles to CR1 also transduces signals that activate the microbicidal mechanisms of the phagocytes, especially when the Fcγ receptor is simultaneously engaged by antibody-coated particles. CR1 on erythrocytes binds circulating immune complexes with attached C3b and C4b and transports the complexes to the liver and spleen. Here, phagocytes remove the immune complexes from the erythrocyte surface, and the erythrocytes continue to circulate. CR1 is also a regulator of complement activation (discussed in the section that follows).

- ***The type 2 complement receptor (CR2, or CD21) functions to stimulate humoral immune responses by enhancing B cell activation by antigen and by promoting the trapping of antigen-antibody complexes in germinal centers.*** CR2 is present on B lymphocytes, FDCs, and some epithelial cells. It specifically binds cleavage products of C3b, called C3d, C3dg, and iC3b (i referring to inactive), which are generated by Factor I–mediated proteolysis (discussed later). On B cells, CR2 is expressed as part of a trimolecular complex that includes two other noncovalently attached proteins called CD19 and CD81 (or TAPA1 [target of antiproliferative antibody-1]). This complex delivers signals to B cells that enhance the responses of B cells to antigen (see Fig. 7.20). On FDCs, CR2 serves to trap iC3b-, C3d-, and C3dg-coated antigen-antibody complexes in germinal centers. The functions of complement in B cell activation are described later.
- ***The type 3 complement receptor, also called MAC-1 (CR3, CD11bC D18), is an integrin that functions as a receptor for the iC3b fragment produced by proteolysis of C3b.*** MAC-1 is expressed on neutrophils, mononuclear phagocytes, mast cells, and NK cells. This member of the integrin family (see Chapter 3) consists of an α chain (CD11b) noncovalently linked to a β chain (CD18) that is identical to the β chains of two closely related integrin molecules, LFA-1 (leukocyte

function–associated antigen 1), and p150,95 (CR4). MAC-1 on neutrophils and monocytes promotes phagocytosis of microbes opsonized with iC3b. In addition, MAC-1 may directly recognize bacteria for phagocytosis by binding to some unknown microbial molecules (see Chapter 4). It also binds to ICAM-1 (intercellular adhesion molecule-1) on endothelial cells and promotes stable attachment of the leukocytes to endothelium, even without complement activation. This binding leads to the recruitment of leukocytes to sites of infection and tissue injury (see Chapter 3).

TABLE 13.8



Receptor	Structure	Ligands	Cell Distribution	Function
Type 1 complement receptor (CR1, CD35)	160–250 kD; multiple CCPRs	C3b > C4b > iC3b	Mononuclear phagocytes, neutrophils, B and T cells, erythrocytes, eosinophils, FDCs	Phagocytosis Clearance of immune complexes Promotes dissociation of C3 convertases by acting as cofactor for cleavage of C3b, C4b
Type 2 complement receptor (CR2, CD21)	145 kD; multiple CCPRs	C3d, C3dg > iC3b	B lymphocytes, FDCs, nasopharyngeal epithelium	Coreceptor for B cell activation Trapping of antigens in germinal centers Receptor for EBV
Type 3 complement receptor (CR3, MAC-1, CD11bCD18)	Integrin, with 165-kD α chain and 95-kD β2 chain	iC3b, ICAM-1; also binds microbes	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis Leukocyte adhesion to endothelium (via ICAM-1)
Type 4 complement receptor (CR4, p150,95, CD11cCD18)	Integrin, with 150-kD α chain and 95-kD β2 chain	iC3b	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis, cell adhesion?

CCPRs, Complement control protein repeats; *EBV,* Epstein-Barr virus; *FDCs,* follicular dendritic cells; *ICAM-1,* intercellular adhesion molecule 1; *MAC,* membrane attack complex; *NK,* natural killer.

- ***The type 4 complement receptor (CR4, p150,95, CD11cCD18) is another integrin with a different α chain (CD11c) and the same β chain as MAC-1.*** It also binds iC3b, and the function of this receptor is probably similar to that of MAC-1. CD11c is abundantly expressed on DCs and is used as a marker for this cell type.
- ***The complement receptor of the immunoglobulin family (CRIg) is expressed on the surface of macrophages in the liver known as Kupffer cells.*** CRIg is an integral membrane

protein with an extracellular region made up of Ig domains. It binds the complement fragments C3b and iC3b and is involved in the clearance of opsonized bacteria and other blood-borne pathogens.

Other receptors include those for C3a, C4a, and C5a, which stimulate inflammation, and for C1q. The proinflammatory effects of C3a, C4a, and C5a are mediated by binding of these fragments to specific receptors on various cell types. The C5a receptor, C5aR1, is the most thoroughly characterized. It is a member of the G protein–coupled receptor family expressed on many cell types, including neutrophils, eosinophils, basophils, monocytes, macrophages, mast cells, endothelial cells, smooth muscle cells, epithelial cells, and astrocytes. The C3a receptor is also a member of the G protein–coupled receptor family. These receptors collaborate with Toll-like receptors (TLRs) in the activation of sentinel cells and phagocytes. The C1q receptor is expressed on phagocytes and may assist in the clearance of apoptotic bodies and protein fibers such as amyloid fibrils, both of which bind C1q.

Regulation of Complement Activation

Activation of the complement cascade and the stability of active complement proteins are tightly regulated to prevent complement activation on normal host cells and to limit the duration of complement activation even on microbial cells and antigen-antibody complexes. Regulation of complement is mediated by several circulating and cell membrane proteins (Table 13.9). Many of these proteins belong to a family called regulators of complement activity (RCA) and are encoded by homologous genes that are located adjacent to one another on chromosome 1 at q3.2. RCA proteins include the cell membrane proteins, decay-accelerating factor (DAF [CD55]), membrane cofactor protein (MCP [CD46]), complement receptor 1 (CR1), and complement receptor 2 (CR2). The circulating RCA proteins in the plasma include Factor H and C4-binding protein (C4BP).

Complement activation needs to be regulated for two reasons. First, low-level complement activation goes on spontaneously, and if such activation is allowed to proceed, the result can be damage to normal cells and tissues. Second, even when complement is activated where needed, such as on microbial cells or antigen-antibody complexes, it needs to be controlled because degradation products of complement proteins can diffuse to adjacent cells and injure them.

TABLE 13.9

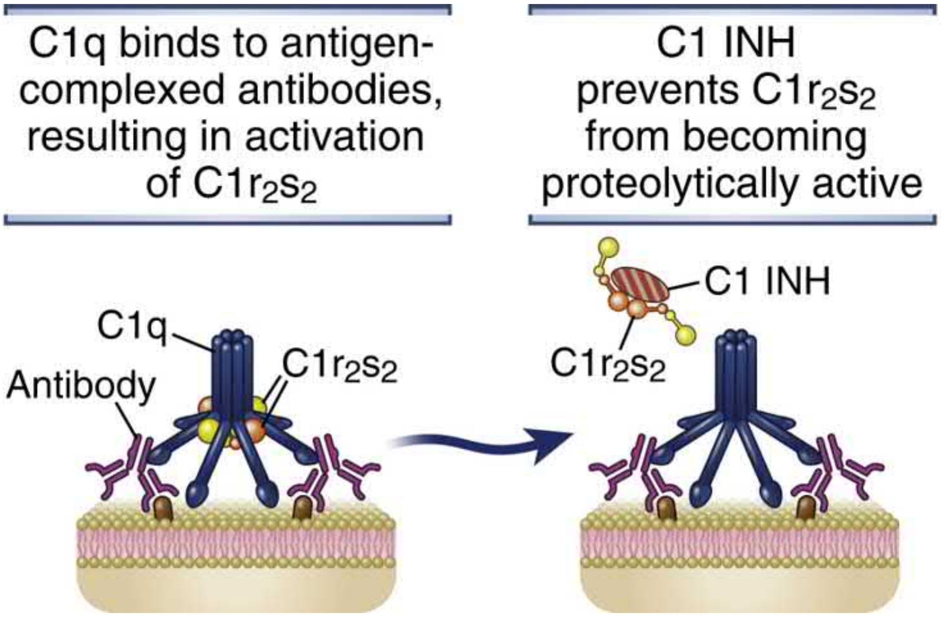
Receptor	Structure	Distribution	Interacts With	Function
C1 inhibitor (C1 INH)	104 kD	Plasma protein; conc. 200 μg/mL	C1r, C1s	Serine protease inhibitor; binds to C1r and C1s and dissociates them from C1q
Factor I	88-kD dimer of 50- and 38-kD subunits	Plasma protein; conc. 35 μg/mL	C4b, C3b	Serine protease; cleaves C3b and C4b by using factor H, MCP, C4BP, or CR1 as cofactors
Factor H	150 kD; multiple CCPRs	Plasma protein; conc. 480 μg/mL	C3b	Binds C3b and displaces Bb
				Cofactor for factor I–mediated cleavage of C3b
C4-binding protein (C4BP)	570 kD; multiple CCPRs	Plasma protein; conc. 300 μg/mL	C4b	Binds C4b and displaces C2
				Cofactor for factor I–mediated cleavage of C4b
Membrane cofactor protein (MCP, CD46)	45–70 kD; four CCPRs	Leukocytes, epithelial cells, endothelial cells	C3b, C4b	Cofactor for factor I–mediated cleavage of C3b and C4b
Decay-accelerating factor (DAF)	70 kD; GPI linked, four CCPRs	Blood cells, endothelial cells, epithelial cells	C4b2a, C3bBb	Displaces C2a from C4b and Bb from C3b (dissociation of C3 convertases)
CD59	18 kD; GPI linked	Blood cells, endothelial cells, epithelial cells	C7, C8	Blocks C9 binding and prevents formation of the MAC

CCPRs, Complement control protein repeats; *conc.,* concentration; *GPI,* glycophosphatidylinositol; *MAC,* membrane attack complex.

Different regulatory mechanisms inhibit the formation of C3 convertases in the early steps of complement activation, break down and inactivate C3 and C5 convertases, and inhibit formation of the MAC in the late steps of complement activation.

- ***The proteolytic activity of C1r, C1s, and MASP2 is inhibited by a plasma protein called C1 inhibitor (C1 INH).*** C1 INH is a serine protease inhibitor (serpin) that mimics the normal substrates of C1r and C1s. If C1q binds to an antibody and begins the process of complement activation, C1 INH becomes a target of the enzymatic activity of the bound $C1r_2$-$C1s_2$. C1 INH is cleaved by and becomes covalently attached to these complement proteins, and, as a result, the $C1r_2$-$C1s_2$ tetramer dissociates from C1q, thus stopping activation by the classical pathway (Fig. 13.13). In this way, C1 INH prevents the accumulation of enzymatically active $C1r_2$-$C1s_2$ in the plasma and limits the time for which active $C1r_2$-$C1s_2$ is available to activate subsequent steps in the complement cascade. Similarly, by inactivating MASP2, C1 INH also dampens the lectin pathway. An autosomal dominant inherited disease called **hereditary angioedema** is due to a deficiency of C1 INH. Clinical manifestations of the disease include intermittent acute accumulation of edema fluid in the skin and mucosa, which causes abdominal pain, vomiting, diarrhea, and potentially life-threatening airway obstruction. In some of these patients, the plasma levels of C1 INH protein are sufficiently reduced (<20% to 30% of normal) that activation of C1 by immune complexes is not properly controlled and increased breakdown of C4 and C2 occurs. The mediators of edema formation in patients with hereditary angioedema include a proteolytic fragment of C2, called C2 kinin, and bradykinin. C1 INH is an inhibitor of other plasma serine proteases besides C1, including kallikrein and coagulation factor XII, both of which can promote increased formation of bradykinin. Recombinant C1 INH is now used to treat patients with this deficiency.

- ***Assembly of the components of C3 and C5 convertases is inhibited by the binding of regulatory proteins of the RCA family to C3b and C4b deposited on cell surfaces*** (Fig. 13.14) **.** If C3b is deposited on the surfaces of normal mammalian cells, it may be bound by several membrane proteins, including MCP (CD46), CR1, and DAF, and the plasma protein Factor H. C4b deposited on cell surfaces is similarly bound by DAF, CR1, MCP, and another plasma protein, C4BP. By binding to C3b or C4b, these proteins competitively inhibit the binding of other components of the C3 convertase, such as Bb of the alternative pathway and C2a of the classical pathway, thus blocking further progression of the complement cascade. (Factor H inhibits binding of only Bb to C3b and is thus a regulator of the alternative but not the classical pathway.) MCP, CR1, and DAF are produced by mammalian cells but not by microbes. Therefore, these regulators of complement selectively inhibit complement activation on host cells and allow complement activation to proceed on microbes. In addition, cell surfaces rich in sialic acid favor binding of the regulatory protein Factor H over the alternative pathway protein Factor B. Mammalian cells express higher levels of sialic acid than most microbes do, which is another reason that complement activation is prevented on normal host cells and permitted on microbes. DAF is a GPI-linked membrane protein expressed on endothelial cells and erythrocytes. A hematopoietic stem cell deficiency of the enzyme required to form such protein-lipid linkages results in a lack of blood cell expression of many GPI-linked membrane proteins, including DAF and CD59 (see later) and causes a disease called **paroxysmal nocturnal hemoglobinuria.** This disease is characterized by recurrent bouts of intravascular hemolysis, at least partly attributable to unregulated complement activation on the surface of erythrocytes. Recurrent intravascular hemolysis in turn leads to chronic hemolytic anemia and venous thrombosis. An unusual feature of this disease is that the causative mutation in the gene that encodes an enzyme responsible for the generation of the GPI anchor is not inherited but is an acquired somatic mutation in hematopoietic stem cells. A rare inherited deficiency of DAF causes small vessel thrombosis and protein-losing enteropathy, which responds well to C5 inhibitor therapy. It does not cause paroxysmal nocturnal hemoglobinuria because CD59 expression is normal.



C1q binds to antigen-complexed antibodies, resulting in activation of $C1r_2s_2$

C1 INH prevents $C1r_2s_2$ from becoming proteolytically active

C1q
Antibody
$C1r_2s_2$
C1 INH
$C1r_2s_2$

FIGURE 13.13 Regulation of C1 activity by C1 inhibitor.C1 inhibitor *(C1 INH)* displaces C1r2s2 from C1q and terminates classical pathway activation.

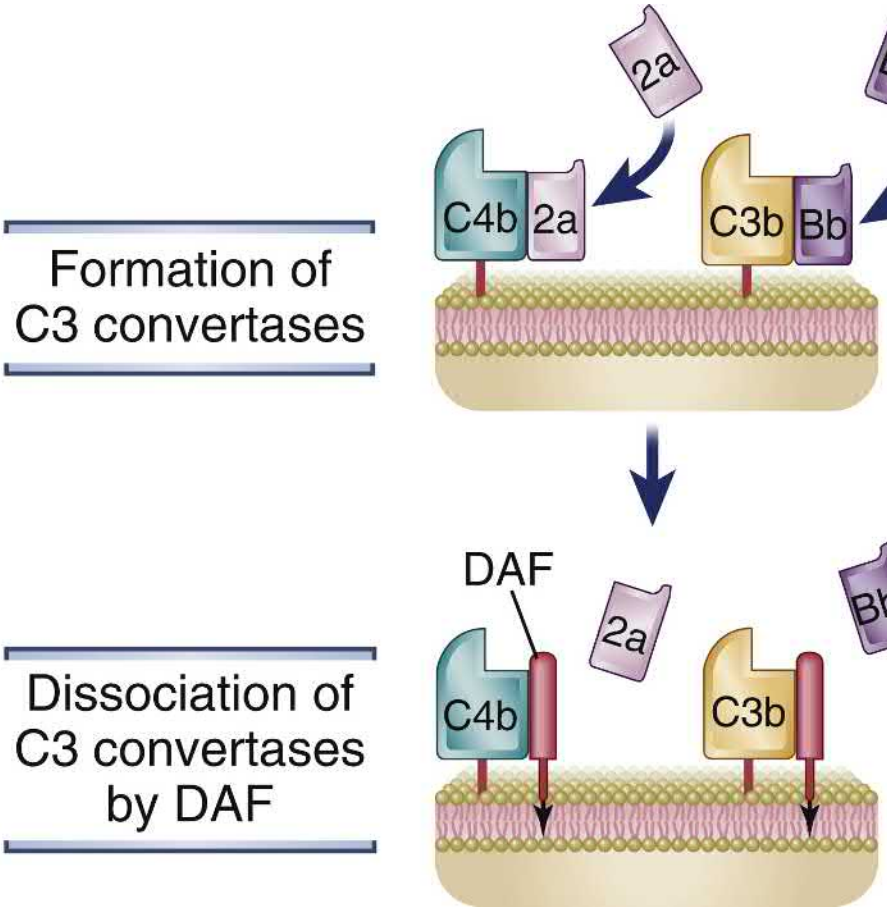

Formation of C3 convertases
2a
Bb
C4b
2a
C3b
Bb
Dissociation of C3 convertases by DAF
DAF
2a
Bb
C4b
C3b

FIGURE 13.14 Inhibition of the formation of C3 convertases. The classical pathway C3 convertase, C4b2a, or the alternative pathway C3 convertase, C3bBb, can be dissociated by the replacement of one component with decay-accelerating factor *(DAF).* Other regulatory proteins, such as membrane cofactor protein *(MCP)* and complement receptor 1 *(CR1),* function similarly to DAF (see text).

- ***Cell-associated C3b is proteolytically degraded by a plasma serine protease called Factor I, which is active only in the presence of regulatory proteins*** (Fig. 13.15). MCP, Factor H, C4BP, and CR1 all serve as cofactors for Factor I–mediated cleavage of C3b (and C4b). Thus, these regulatory host cell proteins promote proteolytic degradation of complement proteins; as discussed earlier, the same regulatory proteins cause dissociation of C3b (and C4b)–containing complexes. Factor I–mediated cleavage of C3b generates the fragments called iC3b, C3d, and C3dg, which do not participate in complement activation but are recognized by receptors on phagocytes and B lymphocytes.

- ***Inflammation induced by C3a and C5a is regulated by the rapid cleavage of their C-terminal arginine residues by plasma carboxypeptidases.*** This results in the generation of C3a des-Arg and C5a des-Arg, each of which has only approximately 10% of the activity of the native forms of these proteins.
- ***Formation of the MAC is inhibited by a membrane protein called CD59.*** CD59 is a GPI-linked protein expressed on many cell types. It works by incorporating itself into assembling MACs after the membrane insertion of C5b-8, thereby inhibiting the subsequent addition of C9 molecules (Fig. 13.16). CD59 is present on normal host cells, where it limits MAC formation, but it is not present on microbes. Formation of the MAC is also inhibited by plasma proteins such as S protein, which functions by binding to soluble C5b,6,7 complexes and thereby preventing their insertion into cell membranes near the site where the complement cascade was initiated. Growing MACs can insert into any neighboring cell membrane besides the membrane on which they were generated. Inhibitors of the MAC in the plasma and in host cell membranes ensure that lysis of innocent bystander cells does not occur near the site of complement activation.

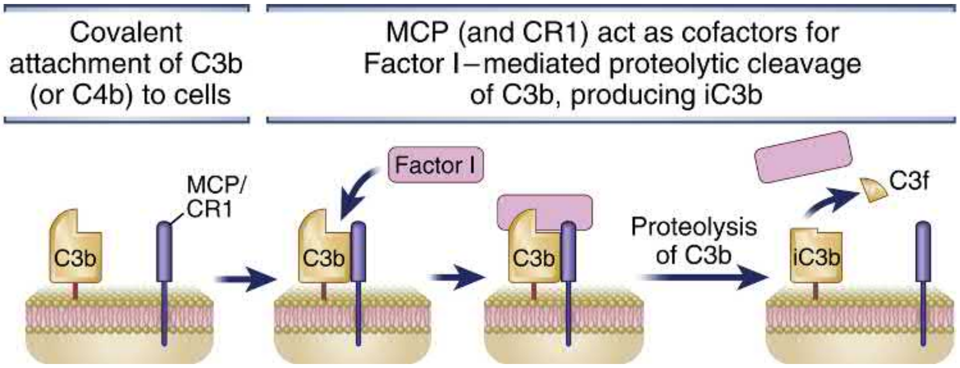

Covalent attachment of C3b (or C4b) to cells
MCP (and CR1) act as cofactors for Factor I–mediated proteolytic cleavage of C3b, producing iC3b
C3b
MCP/ CR1
Factor I
C3b
C3b
Proteolysis of C3b
iC3b
C3f

FIGURE 13.15 Factor I–mediated cleavage of C3b.In the presence of cell membrane–bound cofactors (membrane cofactor protein *[MCP]* or complement receptor 1 *[CR1]*), plasma factor I proteolytically cleaves C3b attached to cell surfaces, leaving an inactive form of C3b (iC3b). Factor H and C4-binding protein can also serve as cofactors for Factor I–mediated cleavage of C3b. The same process is involved in the proteolysis of C4.

Much of the analysis of the function of complement regulatory proteins has relied on in vitro experiments, and most of these experiments have focused on assays that measure MAC-mediated lysis of erythrocytes as an endpoint. On the basis of these studies, a hierarchy of importance for inhibiting complement activation is thought to be CD59 > DAF > MCP; this hierarchy may reflect the relative abundance of these proteins on cell surfaces.

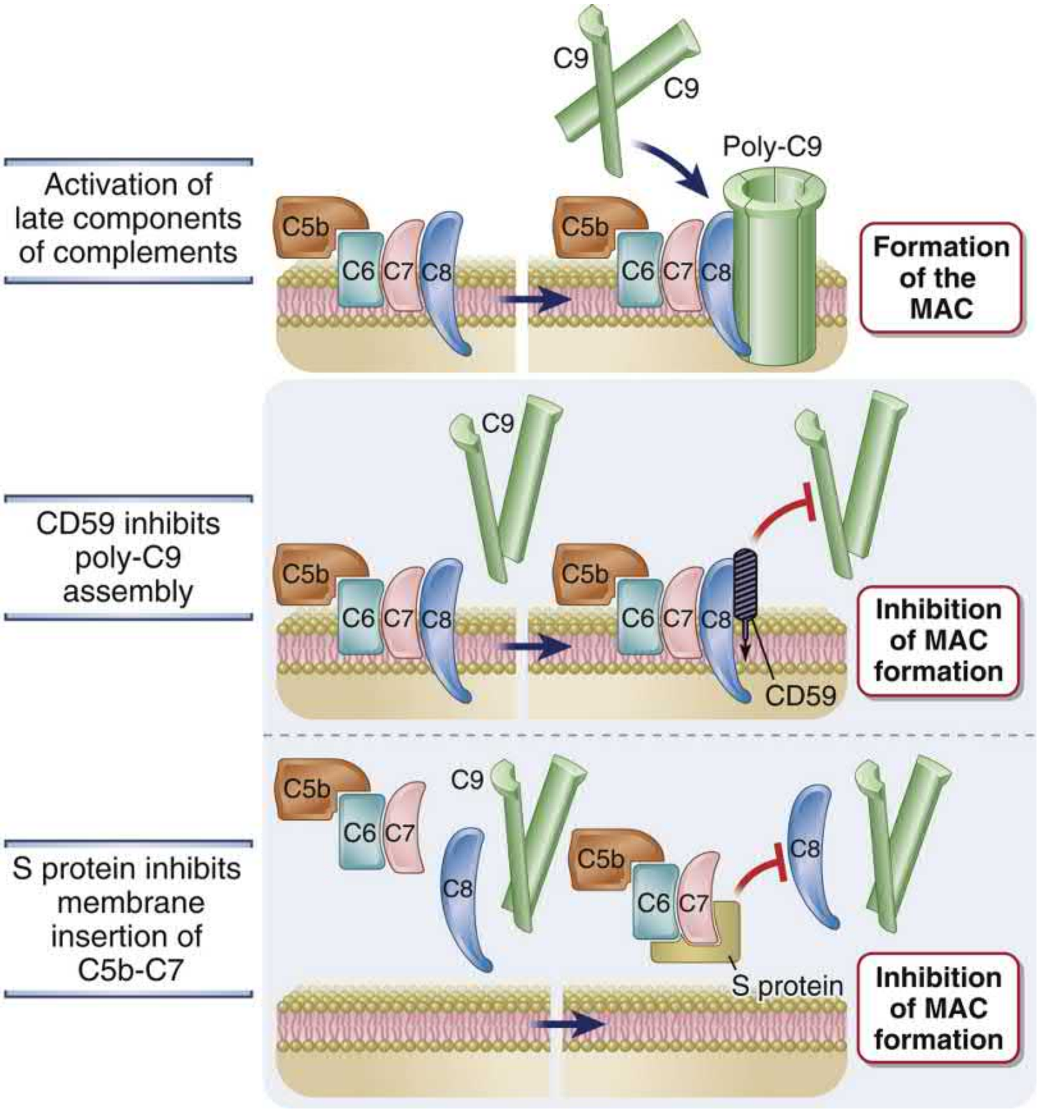

Activation of late components of complements
C5b
C6
C7
C8
C9
C9
Poly-C9
C5b
C6
C7
C8
Formation of the MAC
CD59 inhibits poly-C9 assembly
C9
C5b
C6
C7
C8
C5b
C6
C7
C8
CD59
Inhibition of MAC formation
S protein inhibits membrane insertion of C5b-C7
C5b
C6
C7
C9
C8
C5b
C6
C7
C8
S protein
Inhibition of MAC formation

FIGURE 13.16 Regulation of formation of the membrane attack complex. The membrane attack complex *(MAC)* is formed on cell surfaces as an end result of complement activation. The membrane protein CD59 and S protein in the plasma inhibit formation of the MAC.

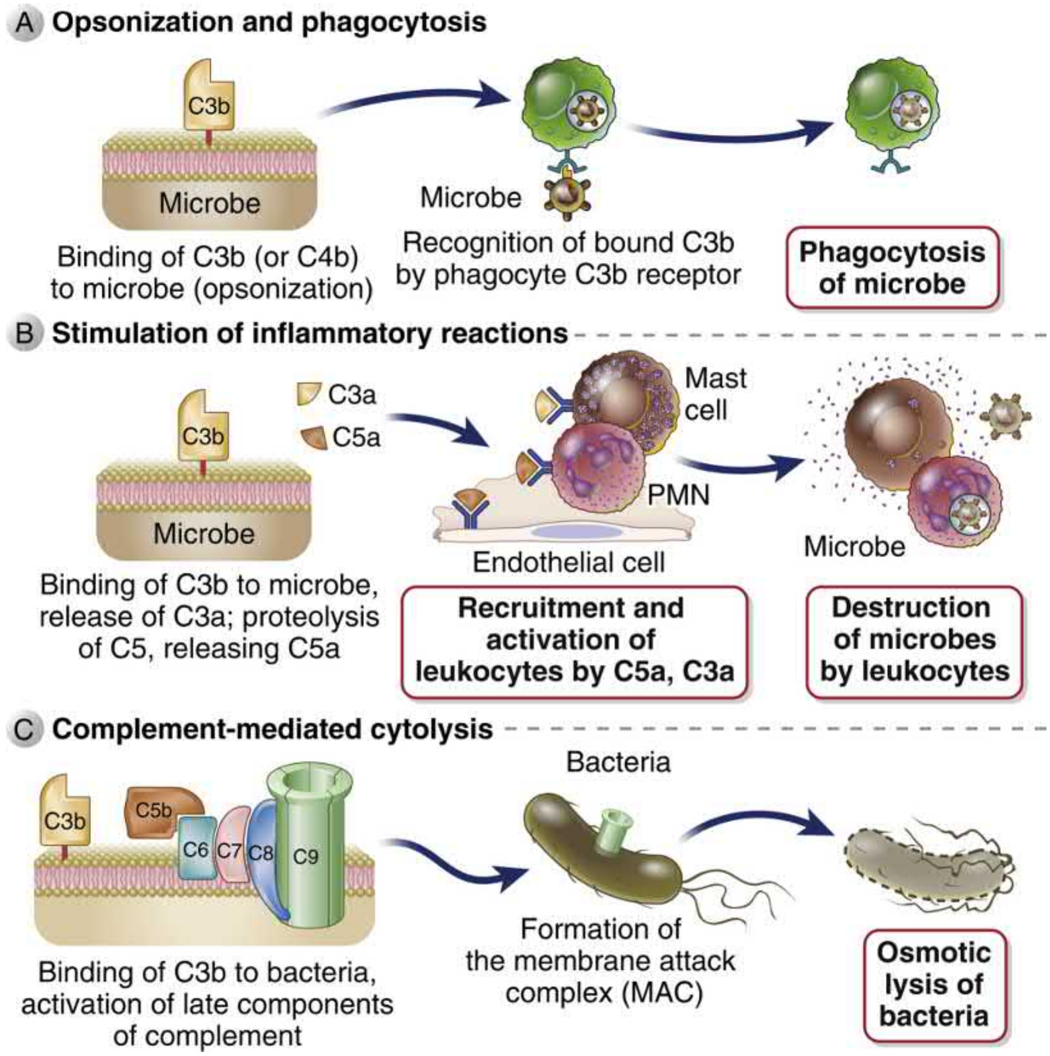

A Opsonization and phagocytosis
C3b
Microbe
Binding of C3b (or C4b) to microbe (opsonization)
Microbe
Recognition of bound C3b by phagocyte C3b receptor
Phagocytosis of microbe
B Stimulation of inflammatory reactions
C3b
C3a
C5a
Microbe
Binding of C3b to microbe, release of C3a; proteolysis of C5, releasing C5a
Mast cell
PMN
Endothelial cell
Recruitment and activation of leukocytes by C5a, C3a
Microbe
Destruction of microbes by leukocytes
C Complement-mediated cytolysis
C3b
C5b
C6
C7
C8
C9
Binding of C3b to bacteria, activation of late components of complement
Bacteria
Formation of the membrane attack complex (MAC)
Osmotic lysis of bacteria

FIGURE 13.17 Functions of complement. The major functions of the complement system in host defense are shown. Cell-bound C3b is an opsonin that promotes phagocytosis of coated cells **(A);** the proteolytic products C5a, C3a, and (to a lesser extent) C4a stimulate leukocyte recruitment and inflammation **(B);** and the membrane attack complex (MAC) lyses cells **(C).**

The function of regulatory proteins may be overwhelmed by excessive activation of complement pathways. We have emphasized the importance of these regulatory proteins in preventing complement activation on normal cells. However, complement-mediated phagocytosis and damage to normal cells are important pathogenic mechanisms in many immunologic diseases (see Chapter 19). In these diseases, large amounts of antibodies may be deposited on host cells, generating enough active complement proteins that the regulatory molecules are unable to control complement activation.

Functions of Complement

The principal functions of the complement system in innate immunity and adaptive humoral immunity are to promote phagocytosis of microbes on which complement is activated, to stimulate inflammation, and to induce the lysis of these microbes. In addition, products of complement activation facilitate the activation of B lymphocytes and the production of antibodies. Phagocytosis, inflammation, and stimulation of humoral immunity are all mediated by the binding of proteolytic fragments of complement proteins to various cell surface receptors, whereas cell lysis is mediated by the MAC. In the following section, we will describe these functions of the complement system and their roles in host defense.

Opsonization and Phagocytosis

Microbes on which complement is activated become coated with C3b, iC3b, or C4b and are phagocytosed by the binding of these proteins to specific receptors on macrophages and neutrophils (Fig. 13.17A) **.** As discussed previously, activation of complement leads to the generation of C3b and iC3b covalently bound to cell surfaces. Both C3b and iC3b act as opsonins by virtue of their ability to specifically bind to receptors on neutrophils and macrophages. C3b and C4b (the latter generated by the classical pathway only) bind to CR1, and iC3b binds to CR3 (Mac-1) and CR4. By itself, CR1 is inefficient at inducing the phagocytosis of C3b-coated microbes, but its ability to do so is enhanced if the microbes are coated with IgG antibodies that simultaneously bind to Fcγ receptors. Macrophage activation by the cytokine IFN-γ also enhances CR1-

mediated phagocytosis. C3b- and iC3b-dependent phagocytosis of microorganisms is a major defense mechanism against infections in innate and adaptive immunity. One example of the importance of complement is host defense against bacteria with polysaccharide-rich capsules, such as pneumococci and meningococci, which is mediated primarily by humoral immunity. IgM antibodies against capsular polysaccharides bind to the bacteria, activate the classical pathway of complement, and cause phagocytic clearance of the bacteria in the spleen. This is why individuals lacking the spleen (e.g. as a result of autosplenectomy in sickle cell anemia patients, or due to surgical removal after traumatic rupture of the spleen or to treat patients with autoimmune hemolytic anemia) are susceptible to disseminated pneumococcal and meningococcal septicemia.

Stimulation of Inflammatory Responses

The proteolytic complement fragments C5a, C4a, and C3a induce acute inflammation by activating mast cells, neutrophils, and endothelial cells (Fig. 13.17B). All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators, such as histamine. These peptides are also called anaphylatoxins because the mast cell reactions they trigger are characteristic of anaphylaxis (see Chapter 20). In neutrophils, C5a stimulates motility, firm adhesion to endothelial cells, and, at high doses, stimulation of the respiratory burst and production of reactive oxygen species. In addition, C5a may act directly on vascular endothelial cells and induce increased vascular permeability and the expression of P-selectin, which promotes neutrophil binding. This combination of C5a actions on mast cells, neutrophils, and endothelial cells contributes to inflammation at sites of complement activation. C5a is the most potent mediator of mast cell degranulation, C3a is approximately 20-fold less potent, and C4a is approximately 2500-fold less.

Complement-Mediated Cytolysis

Complement-mediated lysis of foreign organisms is mediated by the MAC (Fig. 13.17C). Most pathogens have evolved thick cell walls or capsules that impede access of the MAC to their cell membranes. Complement-mediated lysis appears to be critical for defense against only a few pathogens that are unable to resist MAC insertion, such as bacteria of the genus *Neisseria*, which have very thin cell walls.

Other Functions of the Complement System

By binding to antigen-antibody complexes, complement proteins promote the solubilization of these complexes and their clearance by phagocytes. Small numbers of immune complexes are frequently formed in the circulation when an individual mounts a vigorous antibody response to a circulating antigen. If the immune complexes accumulate in the blood, they may be deposited in vessel walls and lead to inflammatory reactions that damage the vessels and surrounding tissue. The formation of immune complexes may require not only the multivalent binding of Ig Fab regions to antigens but also noncovalent interactions of Fc regions of juxtaposed Ig molecules. Complement activation on Ig molecules can sterically block these Fc-Fc interactions, thereby promoting dissolution of the immune complexes. In addition, as discussed earlier, immune complexes with attached C3b bind to CR1 on erythrocytes and the complexes are cleared by phagocytes in the liver.

The C3d protein generated from C3 binds to CR2 on B cells and facilitates B cell activation and the development of humoral immune responses. C3d is generated when complement is activated by an antigen, either directly (e.g., when the antigen is a microbial polysaccharide) or after the binding of antibody. Complement activation results in the covalent attachment of C3b and its cleavage product C3d to the antigen. B lymphocytes can bind the antigen through their Ig receptors and simultaneously bind the attached C3d through CR2, the coreceptor on B cells, thus enhancing antigen-induced signaling in B lymphocytes (see Chapters 7 and

12). Opsonized antigens are also bound by FDCs in the germinal centers of lymphoid organs. FDCs display antigens to B cells in the germinal centers, and this process is important for the selection of high-affinity B cells (see Fig. 12.18). The importance of complement in humoral immune responses is illustrated by the impairment in antibody production and germinal center formation seen in knockout mice lacking C3 or C4 or the CR2 protein.

Complement proteins and natural antibodies contribute to the clearance of apoptotic cells and fragments. Many different host proteins contribute to the clearance of apoptotic cells by macrophages, but the functional importance of complement proteins in this process is revealed in patients with inherited complement deficiencies (discussed next).

Complement Deficiencies

Genetic deficiencies of complement proteins and regulatory proteins are the causes of various human diseases. Inherited and spontaneous deficiencies in many of the complement proteins have been described in humans.

- Genetic deficiencies in classical pathway components, including C1q, C1r, C4, C2, and C3, have been described; C2 deficiency is the most common human complement deficiency. More than 50% of patients with C1q, C2, and C4 deficiencies develop systemic lupus erythematosus. The reason for this association of complement defects and an autoimmune immune complex disease is unknown, but it may be related to inadequate clearance of circulating immune complexes because of defects in complement activation. If normally generated immune complexes are not cleared from the circulation, they may be deposited in blood vessel walls and tissues, where they activate leukocytes by Fc receptor–dependent pathways and produce local inflammation. Complement may also play an important role in the clearance of apoptotic bodies containing fragmented DNA. These apoptotic bodies are likely sources of the nuclear antigens that trigger autoantibody responses in lupus. In addition, complement proteins regulate antigen-mediated signals received by B cells; in their absence, self antigens may not induce B cell tolerance and autoimmunity results. Some patients with C2 or C4 deficiency show increased susceptibility to infections, and others are asymptomatic. Deficiency of C3 is associated with frequent serious pyogenic bacterial infections that may be fatal, illustrating the central role of C3 in opsonization, enhanced phagocytosis, and destruction of these organisms.

- Deficiencies in components of the alternative pathway result in increased susceptibility to meningococcal infections. Factor B and Factor D deficiencies are rare, but X-linked recessive properdin deficiency is more common. Genetic variants of the genes encoding MBL and MASP2 contribute to immunodeficiency in some patients; this is discussed in Chapter 21.
- Deficiencies in the terminal complement components, including C5, C6, C7, C8, and C9, have also been described. Interestingly, the only consistent clinical problem in these patients is a propensity for disseminated infections by *Neisseria* bacteria, including *Neisseria meningitidis* and *Neisseria gonorrhoeae.* As mentioned earlier, complement-mediated bacterial lysis is particularly important for defense against these thin-walled organisms.
- Deficiencies in complement regulatory proteins are associated with abnormal complement activation and a variety of related clinical abnormalities.
    - Deficiencies in C1 INH and DAF cause hereditary angioedema and paroxysmal nocturnal hemoglobinuria, respectively, mentioned earlier.
    - In patients with Factor I deficiency, plasma C3 is depleted as a result of the unregulated formation of fluid-phase C3 convertase (by the normal tickover

mechanism). The clinical consequence is increased infections with pyogenic bacteria.

- Factor H deficiency is rare and is characterized by excess alternative pathway activation, consumption of C3, and glomerulonephritis caused by inadequate clearance of immune complexes and renal deposition of complement by-products.
- A form of hemolytic-uremic syndrome involves defective complement regulation, and the most common mutations in this condition are in the *Factor H* gene. Another gene that is mutated in some patients is the *MCP* gene. In this disease, children present with microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, all triggered by endothelial cell injury caused by hyperactivation of the alternative pathway of complement. Mutant Factor H or MCP binds less well to C3b and C4b on endothelial surfaces, and as a result there is excessive complement activation, leading to the formation of microthrombi and vascular damage.
- Mutations in Factor I or Factor H result in unregulated activation of the alternative complement pathway and kidney diseases collectively called C3 glomerulonephritis. This is similar to the effects of an autoantibody called C3 nephritic factor (C3NeF), which is specific for alternative pathway C3 convertase (C3bBb). C3NeF stabilizes C3bBb and protects the complex from Factor H–mediated dissociation, which results in unregulated activation of C3.
- Specific allelic variants of Factor H are associated with age-related macular degeneration. Excessive inflammation in the absence of complement regulation contributes to the disruption of photoreceptor cells in the macular region and consequent blindness.
- Mutations in the PIG-A (phosphatidylinositol glycosyltransferase-A) gene result in paroxysmal

nocturnal hemoglobinuria, as discussed earlier, as a result of defective GPI anchors for CD59 and DAF.

- Deficiencies in complement receptors include the absence of CR3 and CR4, both resulting from rare mutations in the β chain (CD18) that is shared by the CD11/CD18 family of integrin molecules. The disease caused by this gene defect is called leukocyte adhesion deficiency (see Chapter 21). This disorder is characterized by recurrent pyogenic infections and is caused by inadequate adherence of neutrophils to endothelium at tissue sites of infection and perhaps by impaired iC3b-dependent phagocytosis of bacteria.

Pathologic Effects of the Complement System

Even when it is properly regulated and appropriately activated, the complement system can cause significant tissue damage. Some of the pathologic effects associated with bacterial infections may be due to complement-mediated acute inflammatory responses to infectious organisms. In some situations, complement activation is associated with intravascular thrombosis and can lead to ischemic injury to tissues. For instance, antiendothelial antibodies against vascularized organ transplants and the immune complexes produced in autoimmune diseases may bind to vascular endothelium and activate complement, thereby leading to inflammation and generation of the MAC with damage to the endothelial surface, which favors coagulation. There is also evidence that some of the late complement proteins may activate prothrombinases in the circulation that initiate thrombosis independent of MAC-mediated damage to endothelium

The clearest examples of complement-mediated pathologic conditions are immune complex–mediated diseases. Systemic vasculitis and immune complex glomerulonephritis result from the deposition of antigen-antibody complexes in the walls of blood vessels and kidney glomeruli (see Chapter 19). Complement activated by these deposited immune complexes initiates the acute inflammatory responses that destroy the vessel walls or glomeruli and lead to thrombosis, ischemic damage to tissues, and scarring. Studies with knockout mice lacking the complement proteins C3 or C4 or lacking Fcγ receptors suggest that Fc receptor–mediated leukocyte activation may also cause inflammation and tissue injury as a result of IgG deposition, even in the absence of complement activation.

Two therapeutic agents that target the complement system are currently approved for clinical use. Antibodies against human C5 that block the proteolytic cleavage of C5 are used to treat paroxysmal nocturnal hemoglobinuria, complement-mediated hemolytic uremic syndrome, and neuromyelitis optica and are also being investigated as a therapeutic in a number of chronic inflammatory disorders. Recombinant human C1 INH is used to treat patients with hereditary angioedema.

Evasion of Complement by Microbes

Pathogens have evolved diverse mechanisms for evading the complement system. Some microbes express thick cell walls that prevent the binding of complement proteins, such as the MAC. Gram-positive bacteria and some fungi are examples of microbes that use this relatively nonspecific evasion strategy. A few of the more specific mechanisms used by selected pathogens will be considered here. These evasion mechanisms may be divided into three groups.

- ***Microbes can evade the complement system by recruiting host complement regulatory proteins.*** Many pathogens, in contrast to nonpathogenic microbes, express sialic acids, which can inhibit the alternative pathway of complement by recruiting Factor H, which displaces C3b from Bb. Some pathogens, like schistosomes, *N. gonorrhoeae,* and certain *Haemophilus* species, scavenge sialic acids from the host and enzymatically transfer the sugar to their cell surfaces. Others, including *Escherichia coli K1* and some meningococci, have evolved special biosynthetic routes for sialic acid generation. Some microbes synthesize proteins that can recruit the regulatory protein Factor H to the cell surface. GP41 on human immunodeficiency virus (HIV) can bind to Factor H, and this property of the virus is believed to contribute to virion protection. Many other pathogens have evolved proteins that facilitate the recruitment of Factor H to their cell walls. These include bacteria such as *Streptococcus pyogenes, Borrelia burgdorferi* (the causative agent of Lyme disease), *N. gonorrhoeae,* and *N. meningitidis;* the fungal pathogen *Candida albicans;* and nematodes, such as *Echinococcus granulosus.* Other microbes, such as HIV, incorporate multiple host regulatory proteins into their envelopes. For instance, HIV incorporates the GPI-anchored complement regulatory proteins DAF and CD59 when it buds from an infected cell.

- ***A number of pathogens produce proteins that inhibit different steps in complement activation.*** *E. coli* makes a C1q-binding protein that inhibits the association of C1q, C1r, and C1s. *Staphylococcus aureus* makes a protein called staphylococcal complement inhibitor that binds to and stably inhibits both the classical and alternative pathway C3 convertases and thus inhibits all three complement pathways. Glycoprotein C1 of the herpes simplex virus destabilizes the alternative pathway convertase by preventing its C3b component from binding to properdin. GP160, a membrane protein on *Trypanosoma cruzi,* the causative agent of Chagas disease, binds to C3b and prevents the formation of the C3 convertase and also accelerates its decay. Vaccinia virus complement inhibitory protein structurally resembles human C4BP but can bind to both C4b and C3b and accelerate the decay of both C3 and C5 convertases.
- ***Complement-mediated inflammation also can be inhibited by microbial products.*** *S. aureus* synthesizes a protein called

chemokine inhibitory protein of staphylococci (CHIPS), which is an antagonist of the C5a anaphylatoxin.

Summary

- Humoral immunity is mediated by antibodies and is the effector arm of the adaptive immune system responsible for defense against extracellular microbes and microbial toxins. The antibodies that provide protection against infection may be produced by long-lived plasma cells generated by the first exposure to microbial antigen or by reactivation of memory B cells by the antigen.
- Antibodies block, or neutralize, the infectivity of microbes by binding to the microbes and sterically hindering interactions of the microbes with cellular receptors. Antibodies similarly block the pathologic actions of toxins by preventing binding of the toxins to host cells.
- Antibody-coated (opsonized) particles are phagocytosed by binding of the Fc portions of the antibodies to phagocyte Fc receptors. There are several types of Fc receptors specific for different subclasses of IgG and for IgA and IgE antibodies, and different Fc receptors bind the antibodies with varying affinities. Attachment of antigen-complexed Ig to phagocyte Fc receptors also delivers signals that stimulate the microbicidal activities of phagocytes.
- The complement system consists of serum and membrane proteins that interact in a highly regulated manner to produce biologically active products. The three major pathways of complement activation are the alternative pathway, which is activated on microbial surfaces in the absence of antibody; the classical pathway, which is activated by antigen-antibody complexes; and the lectin pathway, which is initiated by circulating lectins binding to carbohydrates on pathogens. These pathways generate enzymes that cleave the C3 protein, and cleaved products of

C3 become covalently attached to microbial surfaces or antibodies, so subsequent steps of complement activation are limited to these sites. All pathways converge on the common set of late steps, which involve the proteolytic cleavage of C5 and culminate in the formation of a membrane pore.

- Complement activation is regulated by various plasma and cell membrane proteins that inhibit different steps in the cascades.
- The biologic functions of the complement system include opsonization of organisms and immune complexes by proteolytic fragments of C3, followed by binding to phagocyte receptors for complement fragments and phagocytic clearance; activation of inflammatory cells by proteolytic fragments of complement proteins called anaphylatoxins (C3a, C4a, C5a); cytolysis mediated by membrane attack complex formation on cell surfaces; solubilization and clearance of immune complexes; and enhancement of humoral immune responses.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

Antibody Effector Functions and Fc Receptors

∗Berken A, Benacerraf B. Properties of antibodies cytophilic for macrophages. *J Exp Med* . 1966;123:119–144 *(This was the first report that recognized the existence of Fc receptors.)*.

Bournazos S, Wang T.T, Dahan R, et al. Signaling by antibodies: recent progress. *Annu Rev Immunol* . 2017;35:285–311.

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Complement

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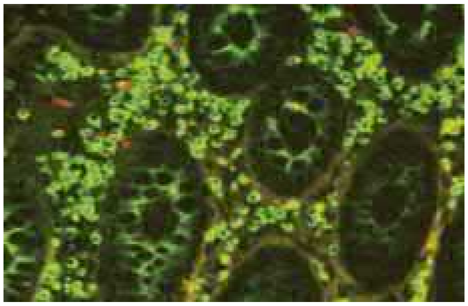
Chapter 14: Specialized Immunity at Epithelial Barriers and in Immune Privileged Tissues



- General Features of Immunity at Epithelial Barriers,
- Immunity In The Gastrointestinal System,
  - Innate Immunity in the Gastrointestinal Tract,
  - Adaptive Immunity in the Gastrointestinal Tract,
  - Regulation of Immunity in the Gastrointestinal Tract by Regulatory T Cells and Cytokines,
  - Oral Tolerance and Oral Vaccines,
  - The Role of the Commensal Microbiome in Immune Regulation,
- Immunity in Other Mucosal Tissues,
  - Immunity in the Respiratory System,
  - Immunity in the Genitourinary System,
- The Cutaneous Immune System,

    Innate and Adaptive Immune Responses in the Skin,

Immune-Privileged Tissues,
    Immune Privilege in the Eye, Brain, and Testis,

Immunity in the Mammalian Fetus and Newborn,
    Immune Privilege of the Mammalian Fetus,
    Passive Immunity in the Fetus and Newborn,

Summary,

Most of our discussion of innate and adaptive immunity so far in this book has covered features and mechanisms of immune responses in any anatomic location in the body. However, the immune system has evolved specialized properties in different parts of the body, especially in epithelial barrier tissues. These features are essential for protection against the types of microbial challenges that are most often encountered at these locations, and they also ensure that we live in harmony with nonpathogenic commensal organisms that colonize epithelial surfaces of the skin and the lumens of mucosal organs (Table 14.1). The collection of the immune cells and molecules serving specialized functions at a particular anatomic location is called a regional immune system. Most of this chapter is devoted to a discussion of these regional immune systems. We end with a consideration of some tissues that do not normally support and may actively suppress immune responses and are said to be immune privileged.

General Features of Immunity at Epithelial Barriers

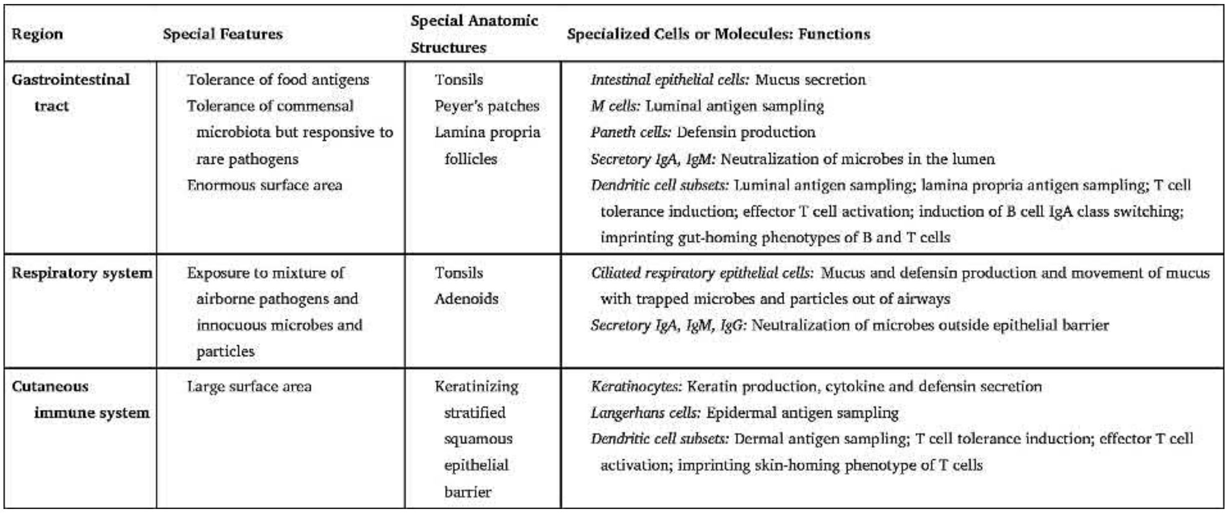
Regional immune systems include the mucosal immune systems, which protect the gastrointestinal, bronchopulmonary, and genitourinary mucosal barriers, and the cutaneous (skin) immune

system. The gastrointestinal immune system is the largest and most complex. The intestinal mucosa of an adult human is estimated to contain approximately 50×10^9 lymphocytes (Table 14.2), and more antibody is made in the intestines than in all other parts of the immune system combined. The dedication of so many immune system resources to the gut reflects the large surface area of the intestinal mucosa, which has evolved to maximize the primary absorptive function of the tissue, but must also resist invasion by trillions of bacteria in the lumen. The skin is also a barrier tissue with vast surface area that must be protected from the environmental microbes that have ready access to the external lining. The total number of lymphocytes in the skin of an adult is estimated to be 20×10^9, about twice the total number of circulating lymphocytes (see Table 14.2). The different physical features of the mucosa (soft, wet, and warm) and the skin (tough, dry, and cool) favor colonization and invasion by different types of microbes. Therefore, it is not surprising that the immune system is specialized in different ways in these two types of tissues.

The immune systems at epithelial barriers share a basic anatomic organization, with an outer epithelial layer that prevents microbial invasion, underlying connective tissue containing various cell types that mediate immune responses to organisms that do invade through the epithelium, and local or more distant draining secondary lymphoid tissues where adaptive immune responses to invading microbes develop. The epithelial barrier may be several layers thick, as in the skin, or a single layer sitting on a basement membrane, as in the intestines. The underlying connective tissue, such as the dermis in the skin and the lamina propria in the gut, contains numerous scattered lymphocytes, dendritic cells (DCs), macrophages, and other cells that mediate innate immune responses and the effector phase of adaptive immune responses. Mucosal tissues also contain unencapsulated but organized secondary lymphoid tissues just under the epithelial barrier, which include B and T lymphocytes, DCs, and macrophages. These collections of immune cells, often called **mucosa-associated**

lymphoid tissue (MALT), are sites of development of some adaptive immune responses specialized for the particular mucosa. Adaptive immune responses in epithelial barrier immune systems are also induced in draining lymph nodes that are located outside the barrier tissues. In skin and mucosal tissues, antigens outside the epithelial barrier are sampled by specialized cells within the epithelium and are delivered to draining lymph nodes or MALT.

Table 14.1



Region	Special Features	Special Anatomic Structures	Specialized Cells or Molecules: Functions
Gastrointestinal tract	Tolerance of food antigens Tolerance of commensal microbiota but responsive to rare pathogens Enormous surface area	Tonsils Peyer's patches Lamina propria follicles	*Intestinal epithelial cells:* Mucus secretion *M cells:* Luminal antigen sampling *Paneth cells:* Defensin production *Secretory IgA, IgM:* Neutralization of microbes in the lumen *Dendritic cell subsets:* Luminal antigen sampling; lamina propria antigen sampling; T cell tolerance induction; effector T cell activation; induction of B cell IgA class switching; imprinting gut-homing phenotypes of B and T cells
Respiratory system	Exposure to mixture of airborne pathogens and innocuous microbes and particles	Tonsils Adenoids	*Ciliated respiratory epithelial cells:* Mucus and defensin production and movement of mucus with trapped microbes and particles out of airways *Secretory IgA, IgM, IgG:* Neutralization of microbes outside epithelial barrier
Cutaneous immune system	Large surface area	Keratinizing stratified squamous epithelial barrier	*Keratinocytes:* Keratin production, cytokine and defensin secretion *Langerhans cells:* Epidermal antigen sampling *Dendritic cell subsets:* Dermal antigen sampling; T cell tolerance induction; effector T cell activation; imprinting skin-homing phenotype of T cells

Table 14.2

Estimated Numbers of Lymphocytes in Different Human Tissues

Spleen	70×10^9
Lymph nodes	190×10^9
Bone marrow	50×10^9
Blood	10×10^9
Skin	20×10^9
Intestines	50×10^9
Liver	10×10^9
Lungs	30×10^9

Regional immune systems contain specialized cell types and molecules that may not be abundant in other sites. The cell types that are restricted to one or more regional immune systems but are not present throughout the immune system include subsets of DCs (e.g., Langerhans cells in the skin), antigen transport cells (e.g., microfold [M] cells in the gut), T lymphocytes (e.g., γδ T cells in epithelia), subsets of B lymphocytes (e.g., immunoglobulin A [IgA]-producing B cells and plasma cells in mucosal tissues), and various innate lymphoid cells (ILCs). The unique anatomic features and cell types in each tissue endow that tissue with special functional characteristics. For example, the sampling of antigens in the gut and their transport to secondary lymphoid organs rely on cell types and routes of lymphatic drainage that are different from what takes place in the skin or internal organs. Furthermore, the MALT structures in various regions of the gut and in other mucosal organs have distinct features.

The effector lymphocytes that are generated in the draining lymph nodes of a particular regional immune system (e.g., skin or small intestine) will enter the blood and preferentially home back to the

same organ (e.g., dermis or lamina propria, respectively). The migration and localization of subsets of lymphocytes to different tissues is in part a result of tissue-specific homing mechanisms that direct these subsets from the blood into particular tissues, which we will discuss in detail later in this chapter.

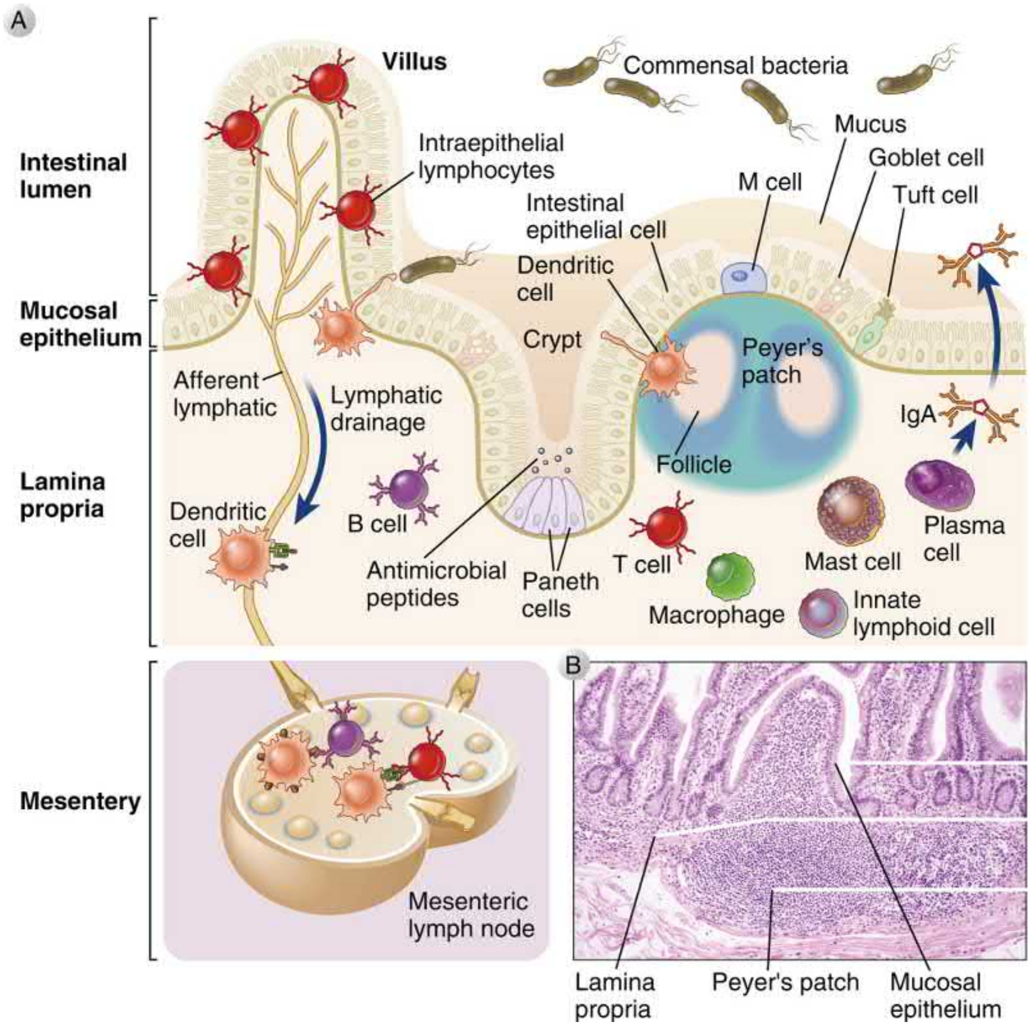

A
Intestinal lumen
Mucosal epithelium
Lamina propria
Mesentery
Villus
Commensal bacteria
Mucus
Goblet cell
Tuft cell
M cell
Intraepithelial lymphocytes
Intestinal epithelial cell
Dendritic cell
Crypt
Peyer's patch
Afferent lymphatic
Lymphatic drainage
IgA
Follicle
Dendritic cell
B cell
Antimicrobial peptides
Paneth cells
T cell
Mast cell
Plasma cell
Macrophage
Innate lymphoid cell
Mesenteric lymph node
B
Lamina propria
Peyer's patch
Mucosal epithelium

FIGURE 14.1 The gastrointestinal immune system. **A,** Schematic diagram of the cellular components of the mucosal immune system in the intestine. The main features include an epithelial barrier covered by secreted mucus, dendritic cells (DCs) and microfold *(M)* cells that sample antigens, Tuft cells that respond to helminths by secreting cytokines, various innate sentinel cells, and lymphocytes in the lamina propria beneath the epithelial layer, organized mucosal-associated lymphoid tissues beneath the epithelial barrier, such as Peyer's patches, draining mesenteric lymph nodes, and plasma cells beneath the epithelium that secrete immunoglobulin A *(IgA),*

which is transported into the lumen. Details of antigen sampling by DCs and M cells, the structure of Peyer's patches, migration of lymphocytes between mucosa and mesenteric lymph nodes, and the secretion and transport of IgA are all described in detail in this chapter. **B,** Photomicrograph of mucosal lymphoid tissue in the human intestine. Similar aggregates of lymphoid tissue are found throughout the gastrointestinal tract.

Regional immune systems have important regulatory functions that prevent unwanted responses to nonpathogenic microbes and foreign substances that are likely to be present at different barriers. The clearest example is the gut-associated immune system, which must suppress responses to commensal bacteria that colonize the intestinal lumen, as well as to foreign food substances, but must respond to pathogenic bacteria, which will be present in much fewer numbers than the commensals. The suppression of immune responses to nonpathogenic organisms and harmless foreign substances is also important in other sites of the body, including the skin, lung, and genitourinary tract, which are not sterile and are constantly exposed to the environment.

With this introduction, we will discuss the details of these various features in different regional immune systems, beginning with the largest.

Immunity in the Gastrointestinal System

The gastrointestinal system, like other mucosal tissues, is composed of a tube-like structure lined by a continuous epithelial cell layer sitting on a basement membrane that serves as a physical barrier to the external environment. Underlying the epithelium is a layer of loose connective tissue called the lamina propria that contains blood vessels, lymphatic vessels, and MALTs (Fig. 14.1).

The submucosa is a dense connective tissue layer between the mucosa and the layers of smooth muscle.

From the perspective of the immunologist, the gastrointestinal tract has several remarkable properties. First, the combined mucosa of the small and large bowel has a total surface area of more than 200 m^2 (the size of a tennis court), most of which is accounted for by the small intestinal villi and microvilli. Second, the lumen of the gut is teeming with microbes, many of which are ingested along with food and most of which are continuously growing in the lumen as commensals in healthy individuals. It is estimated that more than 500 to 1000 different species of bacteria, amounting to approximately 10^{14} cells, live in the mammalian gut, about equal to the total number of all the human cells in the body, or about 10 times the number of nucleated human cells in the body (~90% of human cells are the anucleate red blood cells). There are about 600,000 genes in the human gut microbiome, 30 times more than all the genes in the human genome. These ratios have prompted microbiologists to point out that we are actually more bacterial than human! Humans have evolved to depend on these commensals for several functions, including the degradation of components of our diet that our own cells cannot digest. These commensals also compete with potentially pathogenic microbes in the gut and prevent harmful infections. Although the commensal organisms are beneficial when they are contained on the outside of the gut mucosal barrier, they are potentially injurious if they cross the mucosal barrier and enter the circulation or traverse the bowel wall, especially in immune-compromised individuals. Furthermore, noncommensal pathogenic organisms may become part of the diverse mixture of organisms that make up the gut flora at any time if they are ingested in contaminated food or water. These pathogenic organisms, including bacteria, viruses, protozoa, and helminthic parasites, can cause significant disease, even if they represent a tiny fraction of the microbes in the gut lumen. For health to be maintained, the mucosal immune system must be able to recognize and eliminate these numerically rare pathogens in the presence of great numbers of nonpathogenic microbes. Third, the gut is constantly exposed to nonmicrobial foreign substances in ingested food and normally does not mount immune responses against these.

These challenges have been met by the evolution of a complex set of innate and adaptive immune recognition strategies and effector mechanisms. Overall, intestinal immunity protects us against infections while allowing the persistence of commensal microbes. The gut prevents infections in three major ways:

1. The presence of a thick mucus layer keeps most organisms in the lumen away from the intestinal epithelium.
2. Antibiotic peptides produced by intestinal epithelial cells kill pathogens in the lumen or reduce their entry into the epithelium.
3. IgA, produced by plasma cells in the lamina propria, is transported into the lumen and neutralizes pathogens before they can enter through the epithelium.

The gastrointestinal immune system has to constantly maintain a balance between defense against intestinal pathogens and tolerance to commensals and food antigens. Only some of the mechanisms responsible for this equilibrium are understood. Unfortunately, intestinal infections by pathogenic organisms are frequently not controlled by mucosal immunity and account for millions of deaths each year throughout the world. Many of the features of the gastrointestinal immune system are shared by other mucosal tissues, and we will point out these common features of mucosal immunity.

Innate Immunity in the Gastrointestinal Tract

Intestinal epithelial cells lining the small and large bowel are an integral part of the gastrointestinal innate immune system, involved in responses to pathogens and antigen sampling for delivery to the adaptive immune system in the gut. There are several different types of intestinal epithelial cells, all derived from a common precursor found in the crypts of intestinal glands. Among these are the mucus-secreting goblet cells, which reside at the top of the intestinal villi; antigen-sampling M cells, found in specialized dome structures overlying lymphoid tissues; and anti-bacterial peptide–secreting Paneth cells, found at the bottom of the crypts (see Fig. 14.1). All of these cell types contribute in different ways to the barrier function of the mucosa, as we will discuss later.

Innate immune protection in the gut is mediated in part by the physical and chemical barrier provided by the mucosal epithelial cells and their mucus secretions. Adjacent intestinal epithelial cells are held together by proteins that form tight junctions, which block the movement of microbes between the cells into the lamina propria. In addition, mucosal epithelial cells produce antimicrobial substances, including defensins (see Chapter 4). Several cell types located in the mucosa, including epithelial cells, DCs, macrophages, and ILCs, are capable of mounting inflammatory and antiviral responses. Most of these responses are induced by pattern recognition receptor engagement by microbial ligands, which we discussed in Chapter 4.

Several different extensively glycosylated proteins, called mucins, are secreted by goblet cells and form a viscous physical barrier that prevents microbes from contacting the epithelial lining of the gastrointestinal tract. Mucins contain many different O-linked oligosaccharides and include secreted and cell surface glycoproteins. Most of the intestinal mucus layer is composed of MUC2, which forms a hydrated gel ranging from 300 to 700 μm in thickness. In the small bowel, the mucus forms a single layer, and most of the bacteria are in the outer portion of the mucus. Therefore, bacteria rarely make direct contact with small intestine epithelial cells except at the tips of villi that extend toward the top of the mucus layer. In contrast, colonic mucosa has two layers: an outer less-dense layer that is colonized by bacteria and an inner denser layer that is attached to the epithelium and is bacteria-free. These mucus layers also serve as a matrix for display of antimicrobial substances produced by the epithelial cells. Some mucins act as decoy molecules that can be shed from the epithelial cells and bind to the adhesin proteins that pathogenic bacteria use to attach to host cell membranes. In addition to the secreted mucus, the apical surface of gastrointestinal epithelial cells is coated with membrane-bound mucin proteins, which combine with various glycolipids to form the glycocalyx. This is a dense macromolecular layer, which ranges from 30 to 500 nm in thickness in different locations in the gut. The glycocalyx, like the secreted mucus, serves as a physical barrier to prevent microbial contact.

The mucus barrier of the intestine undergoes turnover and chemical changes in response to various environmental and immune signals, which allow rapid increases in mucosal barrier function. Mucins are constitutively produced by the goblet cells in the gastrointestinal epithelium and by the submucosal glands. They are replaced by newly synthesized molecules every 6 to 12 hours, and many liters of mucus are secreted each day in the adult gut. Several different environmental and immune stimuli can induce dramatic increases in mucin production. These stimuli include cytokines (interleukin-1 [IL-1], IL-4, IL-6, IL-9, IL-13, tumor necrosis factor [TNF], and type I interferons), neutrophil products (such as elastase), and microbial adhesive proteins. These stimuli not only increase mucin gene expression but also alter the glycosylation of the mucins because of induced changes in the expression of glycosyltransferase enzymes. The changes in quantity and glycosylation of mucins are thought to increase barrier function against pathogens.

Defensins produced by intestinal epithelial cells provide innate immune protection against luminal bacteria. Defensins are peptides produced by various cell types in the body that exert lethal toxic effects on microbes by inserting into and causing loss of integrity of their outer phospholipid membranes (see Chapter 4). In the small bowel, the major defensins are the α-defensins, including human defensin 5 (HD5) and HD6, produced constitutively as inactive precursor proteins by Paneth cells located at the base of crypts between microvilli. Active HD5 and HD6 peptides are generated by proteolytic cleavage by trypsin, which is also produced by Paneth cells. In the colon, β-defensins are produced by absorptive epithelial cells in the intestinal crypts, some constitutively and others in response to IL-1 or invasive bacteria. In addition, neutrophil granules are rich in α-defensins, which likely contribute to their antimicrobial functions in the setting of infections of the bowel wall.

Paneth cells and other epithelial cells of the intestine also secrete C-type lectins called regenerating islet-derived proteins (REGIIIs), which block bacterial colonization of the epithelial surface. REGIIIγ in mice and its human homolog REGIIIα bind to gram-positive bacterial peptidoglycan and have bactericidal effects.

Toll-like receptors (TLRs) and cytoplasmic NOD-like receptors (NLRs) expressed by intestinal epithelial cells promote immune responses to invasive pathogens, but also limit inflammatory responses to commensal bacteria. As we discussed in Chapter 4, TLRs and NLRs are cellular receptors that recognize pathogen-associated molecular patterns (PAMPs) produced by microbes and generate signals that promote inflammatory and antiviral responses by the cells. Most luminal bacteria of the gut are nonpathogenic if they are retained outside the epithelial barrier, yet they may express the same array of PAMPs that pathogenic bacteria express, such as lipopolysaccharide, peptidoglycans, CpG DNA, and flagellin. Intestinal epithelial cells express a wide range of TLRs, including TLRs 2, 4, 5, 6, 7, and 9, with different receptors expressed in different regions of the gut. Ligation of some TLRs results in the phosphorylation and reorganization of tight junction proteins causing increased strength of the junctions between epithelial cells. TLR signaling also increases intestinal epithelial motility and proliferation. TLR signaling stimulates the secretion of defensins, REGIII lectins, and IgA, all of which will prevent bacterial transgression of the barrier.

Because inflammatory responses that involve the intestinal epithelial cells can impair barrier function and can lead to bacterial invasion and pathologic inflammation, it is not surprising that stringent control mechanisms have evolved to limit innate immune responses. TLR responses in the gut appear to be regulated in part by levels of expression or compartmentalized expression in only certain sites. Several TLRs, including TLR5, which recognizes bacterial flagellins, and TLR4, which recognizes bacterial endotoxin, are mainly expressed on the basolateral surface of intestinal epithelial cells, where they will be accessible to bacteria that have invaded through the barrier but not to commensal bacteria in the lumen. Similarly, NLR family receptors for flagellins (e.g., NAIP) are expressed in the cytosol of intestinal epithelial cells and will activate inflammatory responses only when pathogenic bacteria or their products gain access to the cytosol. There is also evidence that regulators of TLR signaling inside intestinal epithelial cells maintain a higher threshold for activation of inflammatory responses compared with epithelial cells and DCs in other tissues.

In healthy individuals, DCs and macrophages in the lamina propria of the gut inhibit inflammation and maintain homeostasis. Some intestinal macrophages have a unique phenotype that enables them to phagocytose and kill microbes while secreting antiinflammatory cytokines, such as IL-10. This phenotype is apparently induced in the local mucosal environment by transforming growth factor-β (TGF-β). TLR4 expression on both macrophages and DCs in the lamina propria is lower than in other tissues, and inflammatory gene expression in these cells is often inhibited by microbial products. This may be a mechanism to prevent damaging inflammation in response to commensal bacteria and bacterial products that traverse the epithelial barrier.

ILCs in the intestinal mucosa contribute to immune defense against bacteria and parasites, promote epithelial barrier function, and may suppress responses to commensal bacteria. ILCs do not express T cell antigen receptors (TCRs), but rather respond to local cytokine cues by secreting effector cytokines, and subsets of ILCs exist that secrete cytokines typical of helper T cell subsets (see Chapters 2 and 4). Some of the cytokines that activate ILCs are referred to as alarmins because they are released by epithelial cells in response to injury or microbes and serve as an alarm for the immune system. Most of the ILC3s in the body are found in the gut. In response to IL-1 (an alarmin) and IL-23, ILC3s secrete IL-17 and

IL-22. IL-17 promotes acute inflammatory response to the microbes, and both IL-17 and IL-22 enhance intestinal mucosal barrier function by stimulating production of defensins and by enhancing epithelial tight junction function. Studies in mice show that ILC2s play an important role in intestinal innate immunity against helminths. In response to the alarmin cytokine IL-33 released by stressed or damaged epithelial cells and the epithelium-derived cytokine IL-25, ILC2s secrete IL-5 and IL-13. IL-5 activates eosinophils, which secrete enzymes that degrade the outer integument of helminths, and IL-13 increases mucus production, contributing to expulsion of the worms. A specialized intestinal epithelial cell type called the tuft cell is activated by helminths and other microbes to secrete abundant IL-25, which stimulates ILC2s to secrete IL-13, which in turn stimulates the differentiation of mucus-secreting goblet cells and more tuft cells from intestinal crypt stem cells.

ILC function in the intestinal mucosa is regulated in part by the autonomic nervous system. Experimental evidence from mouse studies shows that ILCs in the gut are stimulated by neuropeptides made by enteric neurons in the gut wall and inhibited by neurotransmitters produced by sympathetic neurons innervating the gut. Most of the evidence for neuronal regulation of ILCs has focused on ILC2s. Many enteric neurons produce neuropeptides, including neuromedin U and vasointestinal peptide, and production of these molecules increases after intestinal helminth infections. Both of these peptides can strongly and rapidly stimulate ILC2s to secrete IL-5 and IL-13, thereby enhancing antihelminthic immunity. Conversely, norepinephrine produced by intestinal sympathetic neurons inhibits ILC2 production of IL-5 and IL-13, which may serve to downregulate the inflammatory response after parasite elimination. Enteric glial cells, which are closely associated with enteric neurons, secrete ligands for a signaling receptor expressed on intestinal ILC3s called RET. The RET ligands derived from the glial cells stimulate IL-22 production by ILC3s, which promotes integrity of the intestinal epithelial barrier.

Mucosa-associated invariant T (MAIT) cells are specific for vitamin B metabolites generated by intestinal bacteria and fungi. Most human MAIT cells are in the liver and thus are in a position to respond to microbes delivered there from the gut via the portal circulation. These cells are described in Chapter 10.

Adaptive Immunity in the Gastrointestinal Tract

The adaptive immune system in the gastrointestinal tract has features that are distinct from adaptive immune systems in other organs.

- ***The major form of adaptive immunity in the gut is humoral immunity directed at microbes in the lumen.*** This function is mediated mostly by dimeric IgA antibodies that are secreted into the lumen of the gut or, in the case of breast-feeding infants, IgA that is secreted into colostrum and mother's milk and ingested by the infant. The antibody in the lumen binds to commensals and pathogens and prevents them from invading through the mucosal epithelial barrier and colonizing the tissues.
- ***The dominance of IgA in mucosal secretions, especially in the gut, is because B cells activated in lymph nodes draining mucosa or in mucosal lymphoid tissues tend to undergo class switching to IgA and IgA-producing B cells tend to home to the gut.*** We will discuss the mechanisms underlying both of these unusual features of mucosal B cells later.
- ***Protective cell-mediated immune responses against microbes in the gut are mediated by helper T cells.*** Th17 cells are the most numerous effector T cell subset in the intestinal mucosa, but Th1 and Th2 cells are also present.
- ***A major mechanism for controlling inflammatory reactions in the gut is the activation of regulatory T cells (Tregs).*** Nowhere else in the body is there such an extensive

commitment of the immune system to maintaining tolerance to foreign antigens, including food antigens and commensal microbial antigens. IL-10–producing Treg subsets are more abundant in MALT than in other lymphoid organs.

We will now discuss the special features of adaptive immunity in the gastrointestinal system, including anatomic organization, antigen sampling, lymphocyte homing and differentiation, and antibody delivery to the lumen.

The Functional Anatomy of the Adaptive Immune System in the Gastrointestinal Tract

Adaptive immune responses in the gut are initiated in discretely organized collections of lymphocytes and antigen-presenting cells closely associated with the mucosal epithelial lining of the bowel and in mesenteric lymph nodes (see Fig. 14.1) **.** Naive lymphocytes are exposed to antigens in these sites and differentiate into effector cells. These gut-associated lymphoid tissues adjacent to the mucosal epithelium are sometimes referred to as GALT, which is the gastrointestinal version of MALT, although the terms are often used interchangeably. The most prominent GALT structures are **Peyer's patches,** found mainly under the epithelium in the distal ileum, but there are many lymphoid follicles, either isolated or in small aggregates, in the lamina propria of the appendix and colon. Peyer's patches have the structure of lymphoid follicles, with germinal centers containing B lymphocytes, T follicular helper cells, follicular dendritic cells, and macrophages. The germinal centers in the follicles are surrounded by IgM- and IgD-expressing naive follicular B cells. A region called the dome is located between the follicles and the overlying epithelium and contains B and T lymphocytes, DCs, and macrophages. Between the follicles are T cell–rich parafollicular areas, similar to lymph nodes, but overall, the ratio of B cells to T cells in GALT is about five times higher than in lymph nodes. Distinct from lymph nodes, GALT structures are not encapsulated, and antigen is delivered directly to these structures, independent of lymphatics. Development of both specialized lymphoid structures, such as Peyer's patches, and isolated follicles in the gut lamina propria requires lymphoid tissue inducer cells, which are a subset of ILC3s that produce the cytokine lymphotoxin-β (LTβ).

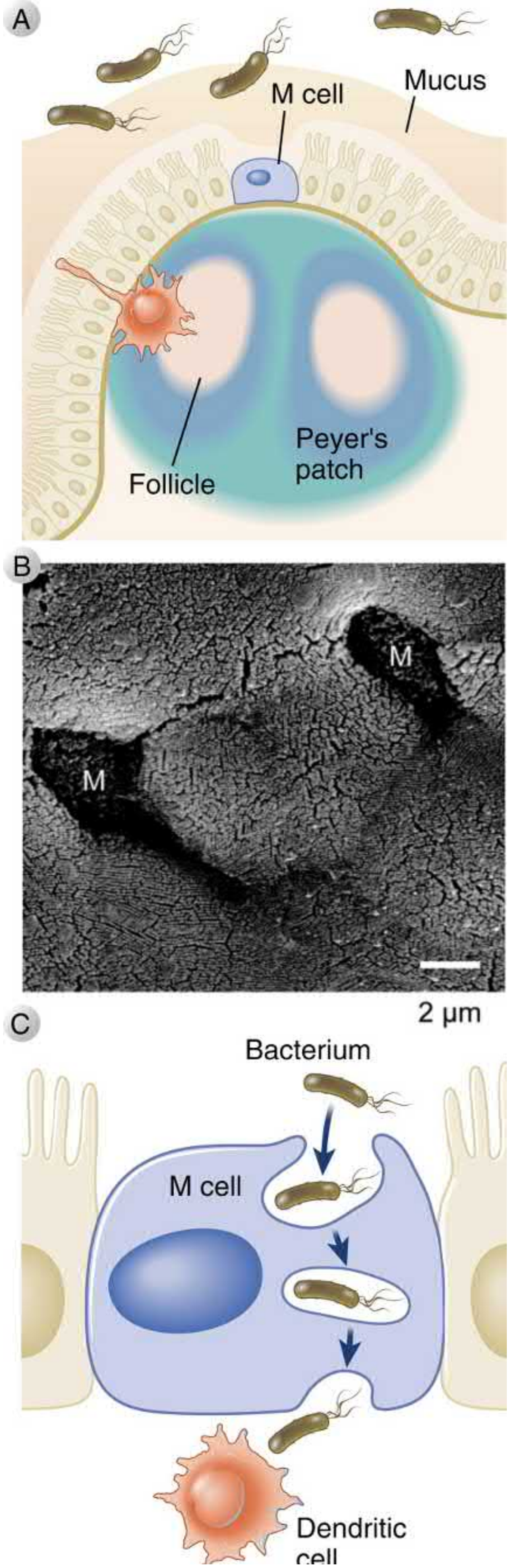

A
Mucus
M cell
Follicle
Peyer's patch
B
M
M
2 µm
C
Bacterium
M cell
Dendritic cell

FIGURE 14.2 M cells in the small intestine.Microfold *(M)* cells are specialized intestinal epithelial cells found in the small bowel epithelium overlying Peyer's patches and lamina propria lymphoid follicles **(A).** Unlike neighboring epithelial cells with tall microvillus borders and primary absorptive functions, M cells have shorter villi. They appear sunken next to absorptive epithelial cells in the scanning electron microscopic image shown in **(B).** M cells engage in transport of intact microbes or molecules across the mucosal barrier into gut-associated lymphoid tissues, where they are handed off to dendritic cells **(C).**

Electron micrograph from Ohno H. Intestinal M cells. *J Biochem.* 2016;159:151–160.

Antigen may be delivered from the lumen to the GALT through specialized cells within the gut epithelium called M cells (Fig. 14.2) **.** M cells are located in regions of the gut epithelium called follicle-associated (or dome) epithelium that overlie the domes of Peyer's patches and other GALT structures. Although M cells and the more numerous absorptive epithelial cells likely arise from a common epithelial precursor, the M cells are distinguishable by a thin glycocalyx, relatively short, irregular microvilli (referred to as microfolds), and large fenestrations in their membranes, all features that enhance the uptake of antigens from the gut lumen. Unlike the absorptive epithelium, the follicle-associated epithelium where M cells are located has a paucity of both mucus-secreting goblet cells and defensin-secreting Paneth cells and reduced ability to transport IgA into the lumen. These features of M cells facilitate their close association with luminal microbial antigens. The main function of M cells is transcellular transport of various substances from the lumen of the intestine across the epithelial barrier to underlying antigen-presenting cells. M cells take up luminal contents efficiently and in various ways, including phagocytosis in a manner similar to macrophages, and vesicular endocytosis or fluid-phase pinocytosis. M cells express various surface molecules that bind microbial structures and mediate their uptake; one example is glycoprotein 2, which binds type I pili on gram-negative bacteria in the gut and mediates uptake and delivery of these bacteria to Peyer's patches. These pathways enable uptake of whole bacteria, viruses, and soluble microbial products. Unlike macrophages or DCs, M cells do not engage in extensive processing of the substances they take up, but rather move the particles and molecules through endocytic vesicles across the cytosol and deliver them by exocytosis at the basolateral membrane to DCs or B cells in the dome regions of underlying Peyer's patches and lamina propria lymphoid follicles. Although M cells play an important role in protective immunity to luminal microbes, some microbes have evolved to take advantage of M cells as a route of invasion through the mucosal barrier. The best described example of this is *Salmonella typhimurium,* which is similar to the human pathogen *Salmonella typhi,* the cause of typhoid fever. M cells express lectins that allow these bacteria to specifically bind and be internalized. The bacteria are toxic to the M cells, producing gaps in the epithelium that promote invasion of more organisms. M cell lectins also may be used by certain enteric viruses to breach the epithelial barrier.

Mesenteric lymph nodes collect lymph-borne antigens from the small and large intestines and are sites of differentiation of effector and regulatory lymphocytes that home back to the lamina propria. There are 100 to 150 of these lymph nodes in the mesentery. Mesenteric lymph nodes serve some of the same functions as GALT, including differentiation of B cells into IgA-secreting plasma cells and the development of effector T cells as well as regulatory T cells. The cells that differentiate in the mesenteric lymph nodes in response to bowel wall invasion by pathogens or commensals often home to the lamina propria (discussed later).

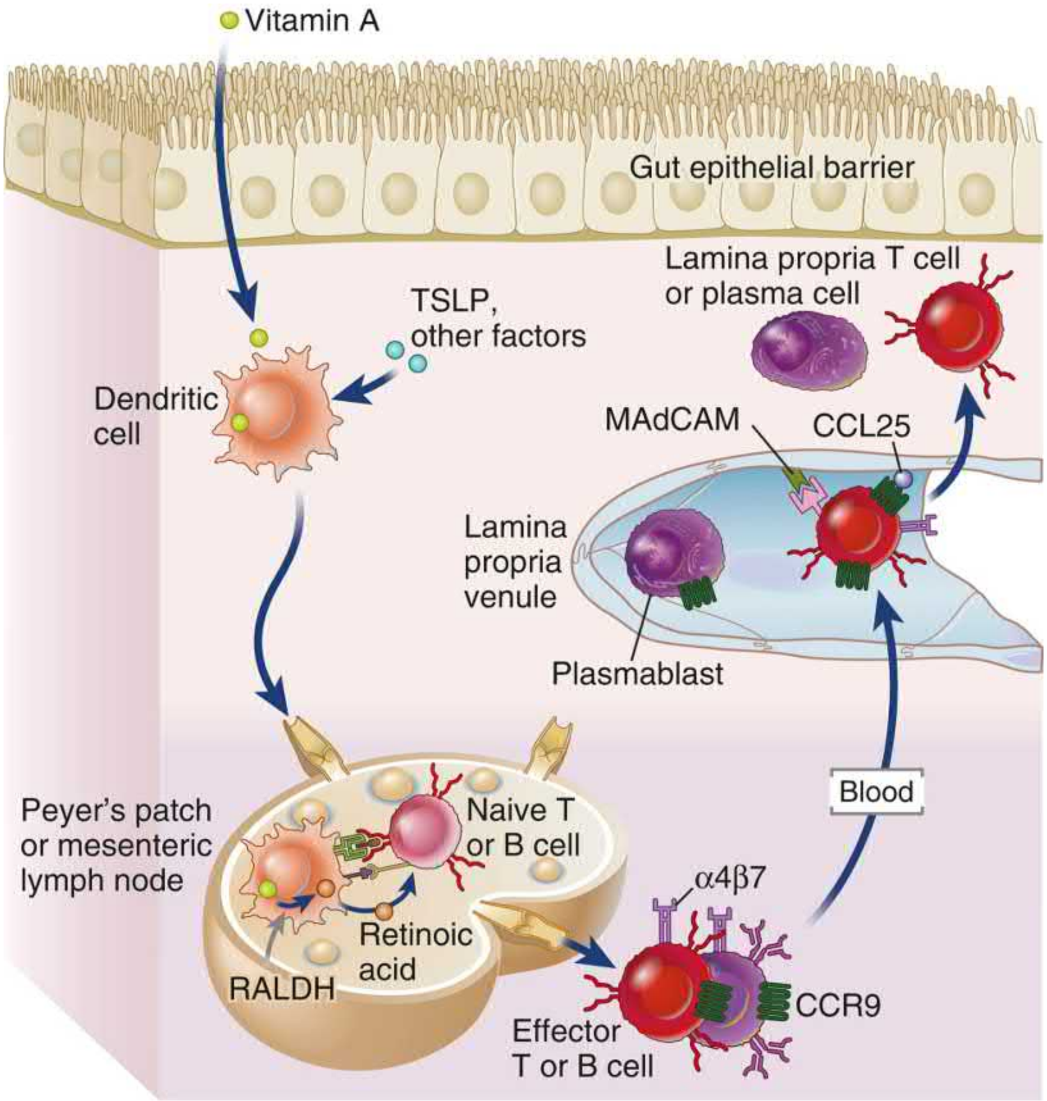

Vitamin A
Gut epithelial barrier
Lamina propria T cell or plasma cell
TSLP, other factors
Dendritic cell
MAdCAM
CCL25
Lamina propria venule
Plasmablast
Blood
Peyer's patch or mesenteric lymph node
Naive T or B cell
α4β7
Retinoic acid
RALDH
CCR9
Effector T or B cell

FIGURE 14.3 Homing properties of intestinal lymphocytes. The gut-homing properties of effector lymphocytes are imprinted in the lymphoid tissues, where they have undergone differentiation by naive precursors. Dendritic cells in gut-associated lymphoid tissues (GALT), including Peyer patches and mesenteric lymph nodes, are induced by cytokines such as thymic stromal lymphopoietin *(TSLP)* and other factors to express retinaldehyde dehydrogenase *(RALDH),* which converts dietary vitamin A into retinoic acid. When naive B or T cells are activated by antigen

in GALT, they are exposed to retinoic acid produced by the dendritic cells, and this induces the expression of the chemokine receptor CCR9 and the integrin $\alpha_4\beta_7$ on the plasmablasts and effector T cells that arise from the naive lymphocytes. The effector lymphocytes enter the circulation and home back into the gut lamina propria because the chemokine CCL25 (the ligand for CCR9) and the mucosal addressin cell adhesion molecule 1 *(MAdCAM -1)* (the ligand for $\alpha_4\beta_7$) are displayed on lamina propria venular endothelial cells.

Lingual and palatine tonsils are unencapsulated lymphoid structures located beneath stratified squamous epithelial mucosa in the base of the tongue and oropharynx, respectively, and are sites of immune responses to microbes in the oral cavity. These tonsils, together with nasopharyngeal tonsils (also called adenoids), form a ring of lymphoid tissue called Waldeyer's ring. The bulk of the tonsillar tissue is composed of lymphoid follicles, usually with prominent germinal centers. The lingual and palatine tonsils are separated from the microbe-rich oral cavity by multiple layers of squamous epithelial cells, rather than the single columnar epithelial cell layer that separates the intestinal lumen from other GALT. There are numerous narrow and deep invaginations of the surface squamous epithelium, called crypts, which grow into the tonsillar follicular tissue. The lingual and palatine tonsils respond to infections of the epithelial mucosa by significant enlargement and vigorous, mainly IgA, antibody responses. Typically, tonsillar enlargement is caused by infection with streptococci and the Epstein-Barr virus, often in children.

Effector lymphocytes that are generated in the GALT and mesenteric lymph nodes are imprinted with selective integrin- and chemokine receptor–dependent gut-homing properties, and they circulate from the blood back into the lamina propria of the gut (Fig.

14.3) . The functions of the gastrointestinal immune system depend on a large number of T cells and antibody-secreting cells that are able to recirculate back into the lamina propria and respond rapidly to pathogens. Both effector T cells and IgA-secreting B cells acquire this gut-homing phenotype because of changes in adhesion molecules and chemokine receptors that are acquired during lymphocyte activation in the GALT or gut-draining lymph nodes. The major integrin on gut-homing B and T lymphocytes is $\alpha_4\beta_7$, which binds to the MAdCAM-1 protein expressed on postcapillary venular endothelial cells in the gut lamina propria. Gut homing requires the chemokine receptor CCR9 on the B and T lymphocytes and its chemokine ligand CCL25, which is produced by intestinal epithelial cells. The combined expression of MAdCAM-1 on endothelium and CCL25 in tissues is restricted to the gut. Homing of IgA-producing cells to the colon also requires CCR10 expression and the chemokine CCL28, but this is not a gut-specific pathway because CCL28 is expressed by epithelial cells in other mucosal tissues, such as the lung and genitourinary tract. Blocking monoclonal antibodies that are specific for the α_4 chain of $\alpha_4\beta_7$ have been used to treat patients with inflammatory bowel disease (IBD) on the basis of the knowledge that effector T cells use this integrin to enter gut tissues.

The gut-homing phenotype of IgA-producing B cells and effector T cells is imprinted by DCs through the action of retinoic acid during the process of T cell activation (see Fig. 14.3) . In addition to promoting naive T cell differentiation into effector T cells and naive B cell differentiation into IgA antibody–secreting cells (discussed later), DCs in GALT and mesenteric lymph nodes also provide signals that lead to the expression of the $\alpha_4\beta_7$ integrin and CCR9 on these effector cells. The induction of these homing molecules depends on secretion of retinoic acid by the DCs. Gut lymphoid tissues are exposed to dietary vitamin A, and DCs in GALT and mesenteric lymph nodes express retinaldehyde dehydrogenase (RALDH), the enzyme needed for retinoic acid synthesis from vitamin A, whereas DCs in other tissues do not. In addition, intestinal epithelial cells express RALDH and can synthesize retinoic acid. How retinoic acid induces expression of gut-homing molecules is not known. Consistent with these properties of the intestinal immune system, it is known that oral vaccination favors the expansion of gut-homing IgA-producing B cells as compared with intradermal immunization.

The lamina propria contains diffusely distributed effector lymphocytes, DCs, and macrophages and is the site of the effector phase of gastrointestinal adaptive immune responses. As discussed previously, effector lymphocytes generated in Peyer's patches, other GALT structures, and mesenteric lymph nodes home back into the lamina propria. In this location, T cells can respond to invading pathogens, and B cells can secrete antibodies that are transported into the lumen and neutralize pathogens before they invade.

Humoral Immunity in the Gastrointestinal Tract

The major function of humoral immunity in the gastrointestinal tract is to neutralize luminal microbes, and this function is mediated mainly by IgA produced in the lamina propria and transported across the mucosal epithelium into the lumen. Smaller quantities of IgG and IgM are also secreted into the gut lumen. Within the lumen, the antibodies bind to microbes and toxins and neutralize them by preventing their binding to host cells. This form of humoral immunity, sometimes called secretory immunity, has evolved to be particularly prominent in mammals. Studies in mice indicate that IgA responses are made to antigens expressed on only a small fraction of all the commensal species in the gut, and these are largely bacteria in the small intestine and not the colon. In addition to specifically binding microbes, glycans in the secretory component of IgA (discussed later) can bind to bacteria and reduce their motility, thereby preventing them from reaching the epithelial barrier. Patients with selective IgA deficiency often present with gastrointestinal and respiratory infections (see Chapter 21). Beyond a role in protective immunity, IgA may also facilitate small intestinal colonization by "helpful" commensals, especially

Bacteroides species. One mechanism by which secreted IgA may promote survival of certain bacterial species is that the glycans on the Fc regions and the secretory piece serve as carbon sources for the microbes. Antibody responses to antigens encountered by ingestion are typically dominated by IgA, and secretory immunity is the mechanism of protection induced by oral vaccines such as the polio vaccine.

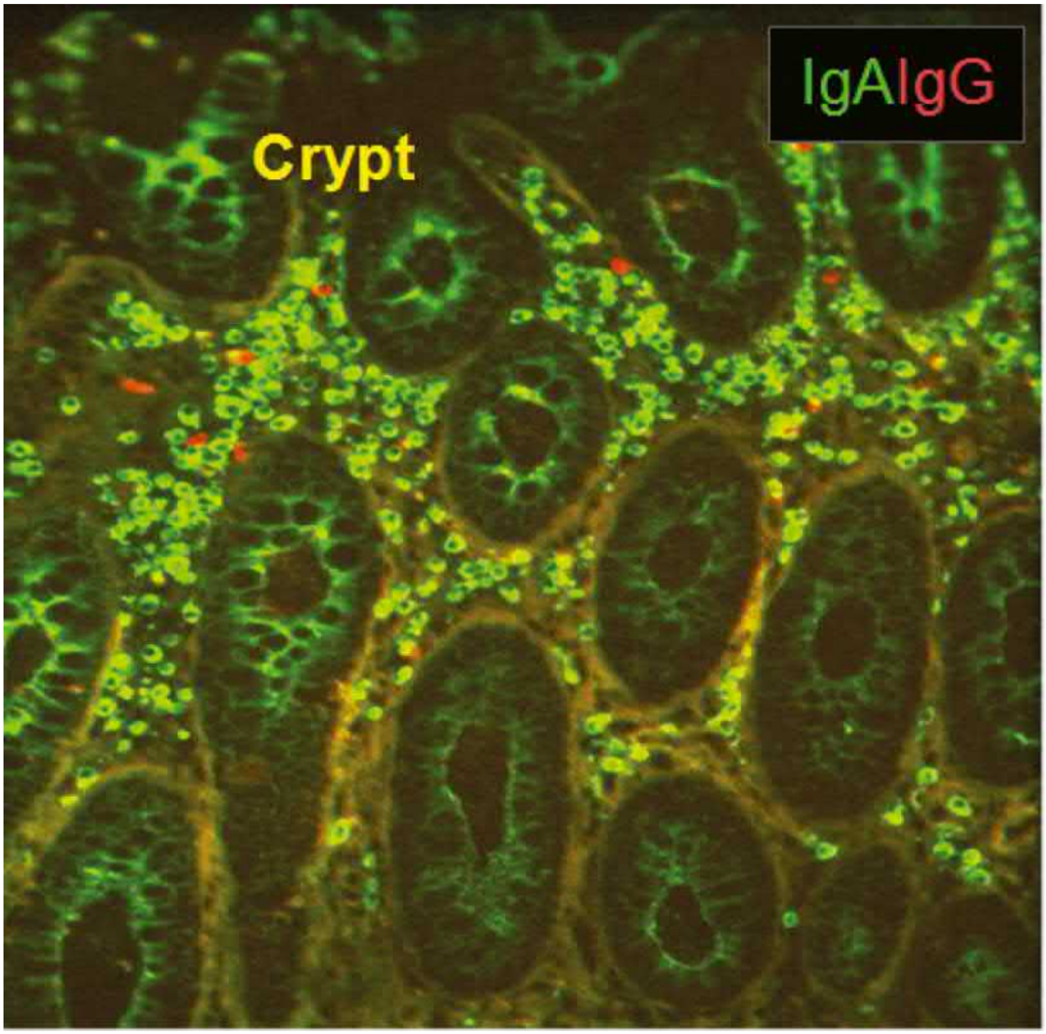


FIGURE 14.4 IgA-secreting plasma cells in the intestine.The abundance of immunoglobulin A *(IgA)*-producing plasma cells *(green)* in colon mucosa compared with IgG-secreting cells *(red)* is shown by immunofluorescence staining. IgA that is being transcytosed by the crypt epithelial cells is visualized in their cytoplasm *(green fluorescent stain)*.

From Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. *J Pediatr.* 2010;156[Suppl. 1]:S8–S16.

IgA is produced in higher amounts than any other antibody isotype. It is estimated that a normal 70-kg adult secretes about 2 g of IgA per day, which accounts for 60% to 70% of the total production of antibodies. This tremendous output of IgA is because of the large number of IgA-producing plasma cells in the GALT, which by some estimates account for 80% of all the antibody-producing plasma cells in the body (Fig. 14.4). Because IgA synthesis occurs mainly in mucosal lymphoid tissue and most of the locally produced IgA is efficiently transported into the mucosal lumen, this isotype constitutes less than one-quarter of the antibody in plasma and is a minor component of systemic humoral immunity compared with IgG.

Several unique properties of the gut environment result in selective development of IgA-secreting cells that stay in the gastrointestinal tract or, if they enter the circulation, home back to the lamina propria of the intestines. The result is that IgA-secreting cells efficiently accumulate next to the epithelium that will take up the secreted IgA and transport it into the lumen.

The abundance of intestinal plasma cells that produce IgA is due in part to selective induction of IgA isotype switching in B cells in GALT and mesenteric lymph nodes. IgA class switching in the gut can occur by T-dependent and T-independent mechanisms (Fig. 14.5). Studies in mice suggest that most of the IgA secreted into the lumen is produced by T-independent mechanisms. In both T-dependent and T-independent IgA class switching, two essential steps are cytokine-induced transcription through the genomic IgA locus, which opens up access to the enzymes needed for switch recombination, and the induced expression of activation-induced deaminase (AID), the enzyme that mediates switch recombination (see Chapter 12). TGF-β is the major cytokine required for both T-dependent and T-independent IgA isotype switching in the gut and in other mucosal tissues. In the gut, TGF-β is produced by epithelial cells and DCs in GALT. Furthermore, GALT DCs express the αvβ8 integrin, which is required for activation of TGF-β. In T-dependent IgA class switching, AID is induced in B cells by CD40 signaling, which is activated by CD40-ligand on T follicular helper (Tfh) cells binding to CD40 on B cells that present antigen to the Tfh cells (see Fig. 14.5A). In T-independent IgA class switching, AID expression in B cells is induced by membrane and secreted forms of the cytokines APRIL (a proliferation-inducing ligand) and BAFF (B cell–activating factor), which are structurally related to CD40L and bind to a CD40-related receptor on B cells called TACI (transmembrane activator and calcium-modulating cyclophilin-ligand interactor). APRIL and BAFF are produced by GALT DCs, and intestinal epithelial cells produce APRIL in response to TLR ligands made by commensal bacteria. Intestinal epithelial cells also produce TSLP (thymic stromal lymphopoietin) in response to TLR signals, and TSLP stimulates additional APRIL production by GALT DCs. TLR ligands made by commensal bacteria in the gut also increase expression of inducible nitric oxide synthase in DCs, leading to nitric oxide production. Nitric oxide is thought to promote both T-dependent and T-independent IgA class switching by enhancing the expression of TGF-β receptors and APRIL. Intestinal B cell IgA production is at least partly dependent on the vitamin A metabolite all-trans retinoic acid, which is made by intestinal epithelial cells and GALT DCs, although the mechanisms by which retinoic acid promotes IgA production are not known. Retinoic acid is also important in B cell homing to the gut, as discussed earlier. There is an abundance of TGF-β and retinoic acid within the GALT and mesenteric lymph nodes compared with nonmucosal lymphoid tissues such as spleen and skin-draining lymph nodes, largely accounting for the propensity of B cells in the GALT to switch to IgA production.

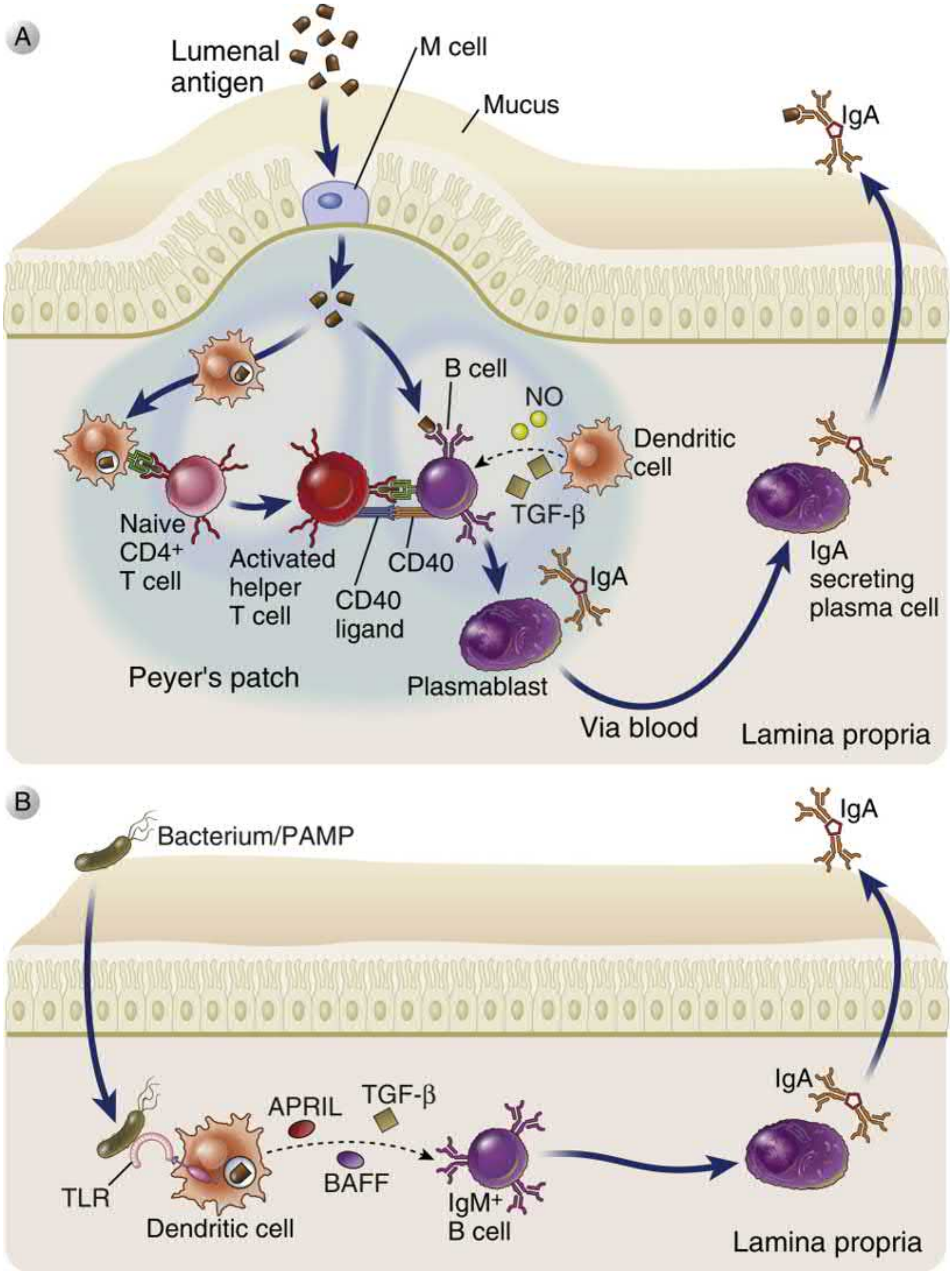

A
Lumenal antigen
M cell
Mucus
IgA
B cell
NO
Dendritic cell
TGF-β
Naive CD4+ T cell
Activated helper T cell
CD40
CD40 ligand
IgA
IgA secreting plasma cell
Peyer's patch
Plasmablast
Via blood
Lamina propria
B
Bacterium/PAMP
IgA
APRIL
TGF-β
IgA
TLR
BAFF
Dendritic cell
IgM+ B cell
Lamina propria

FIGURE 14.5 IgA class switching in the gut.Immunoglobulin A *(IgA)* class switching in the gut occurs by both T-dependent and T-independent mechanisms. **A,** In T-dependent IgA class switching, dendritic cells in the subepithelial dome of Peyer's patches capture bacterial

antigens delivered by microfold *(M)* cells and migrate to the interfollicular zone, where they present antigen to naive $CD4^+$ T cells. The activated T cells differentiate into helper T cells with a T follicular helper phenotype and engage in cognate interactions with antigen-presenting IgM^+ B cells that have also taken up and processed the bacterial antigen. B cell class switching to IgA is stimulated through T cell CD40L binding to B cell CD40, together with the action of transforming growth factor-β *(TGF-β).* This T cell–dependent pathway yields high-affinity IgA antibodies. **B,** T-independent IgA class switching involves dendritic cell activation of IgM^+ B cells, including B-1 cells. Toll-like receptor (TLR) ligand–activated dendritic cells secrete cytokines that induce IgA class switching, including BAFF, APRIL, and TGF- β. This T cell–independent pathway yields relatively low-affinity IgA antibodies to intestinal bacteria. The molecular mechanisms of class switching are described in Chapter 12. *APRIL,* A proliferation-inducing ligand; *BAFF,* B cell–activating factor; *PAMP,* pathogen-associated molecular pattern.

IgA production in the gastrointestinal tract is further enhanced by selective gut-homing properties of IgA-producing cells that arise in GALT and mesenteric lymph nodes (see Fig. 14.3) **.** Some of the IgA that is transported across the intestinal epithelium may be produced by plasma cells that differentiated and remained within underlying GALT follicles. However, IgA-secreting plasma cells are widely dispersed in the lamina propria of the gastrointestinal tract, not just in lymphoid follicles. As discussed earlier, activated B cells that undergo isotype switching into IgA-producing cells in the GALT and mesenteric lymph nodes may enter the systemic circulation and then selectively home back to the intestinal lamina propria, where they may reside as plasma cells.

Secreted IgA is transported through epithelial cells into the intestinal lumen by an Fc receptor called the poly-Ig receptor (Fig. 14.6) **.** The IgA secreted by plasma cells in the lamina propria is in the form of a dimer that is held together by the coordinately produced J chain, which is covalently bound by disulfide bonds to the Fc regions of the α heavy chains of two IgA molecules. Mucosal plasma cells produce abundant J chain, more than plasma cells in nonmucosal tissues, whereas serum IgA is usually a monomer lacking the J chain. The dimeric IgA must be transported from the lamina propria across the epithelium into the lumen. This function is mediated by the poly-Ig receptor, an integral membrane glycoprotein with five extracellular Ig domains. IgM produced by lamina propria plasma cells is also a polymer (pentamer) associated covalently with the J chain, and the poly-Ig receptor also transports IgM into intestinal secretions. This is why this receptor is called the poly-Ig receptor. This receptor is synthesized by mucosal epithelial cells and is expressed on the basal and lateral surfaces of epithelial cells. Its production can be increased by inflammatory stimuli.

Dimeric IgA (and pentameric IgM) secreted by plasma cells in the lamina propria bind to the poly-Ig receptor on the basolateral surface of mucosal epithelial cells through a domain of the J chain (see Fig. 14.6). The antibody-receptor complex is endocytosed into the epithelial cell, and unlike other endosomes that typically traffic to lysosomes, poly Ig-receptor–containing vesicles are directed to and fuse with the apical (luminal) plasma membrane of the epithelial cell. On the apical cell surface, the poly-Ig receptor is proteolytically cleaved, its transmembrane and cytoplasmic domains are left attached to the epithelial cell, and the extracellular domain of the receptor, carrying the IgA molecule, is released into the intestinal lumen. This process of IgA transport across the epithelium is called transcytosis. The cleaved part of the poly-Ig receptor, called the secretory component, remains associated with the dimeric IgA in the lumen. It is thought that the bound secretory component protects IgA (and IgM) from proteolysis by proteases present in the intestinal lumen, and these antibodies are therefore able to serve their function of neutralizing microbes and toxins in the lumen.

IgG is present in intestinal secretions at levels equal to those of IgM but lower than those of IgA. In some mucosal secretions (i.e., in the rectum, genitourinary tract, and airways), IgG levels are quite high. The transport of IgG into mucosal secretions may be mediated by transcytosis via the neonatal Fc receptor (FcRn), which we discussed in Chapters 5 and 13.

IgA produced in lymphoid tissues in the mammary gland is secreted into colostrum and mature breast milk through poly-Ig receptor–mediated transcytosis and mediates passive mucosal immunity in breast-fed children. The human lactating mammary gland contains a large number of IgA-secreting plasma cells, and the mammary gland epithelium can store large quantities of secretory IgA. The plasma cells in the breast may originate in various MALTs. They home to the breast because most IgA plasmablasts express CCR10, regardless of the lymphoid tissues in which they were generated, and the breast tissues express CCL28, the chemokine that binds CCR10. During breast-feeding, a child ingests a significant quantity of maternal IgA, which provides broad polymicrobial protection in the infant's gut. Moderate amounts of IgG and IgM are also secreted into breast milk and contribute to the passive immunity of breast-fed children. Many epidemiologic studies have shown that breast-feeding significantly reduces the risk for diarrheal disease and sepsis, especially in developing countries, and this correlates with the presence of secretory IgA in breast milk specific for enterotoxic species of bacteria including *Escherichia coli* and *Campylobacter.*

T Cell–Mediated Immunity in the Gastrointestinal Tract

T cells play important roles in protection against microbial pathogens in the gastrointestinal system and in regulating responses to food and commensal antigens. Furthermore, T cells contribute to inflammatory diseases in the gastrointestinal tract. As in other parts of the body, T cell immunity in the gut involves different subsets of T cells and is influenced in various ways by antigen-presenting DCs, which also belong to different subsets. In this section, we will discuss important features of T cell and DC functions in the intestines.

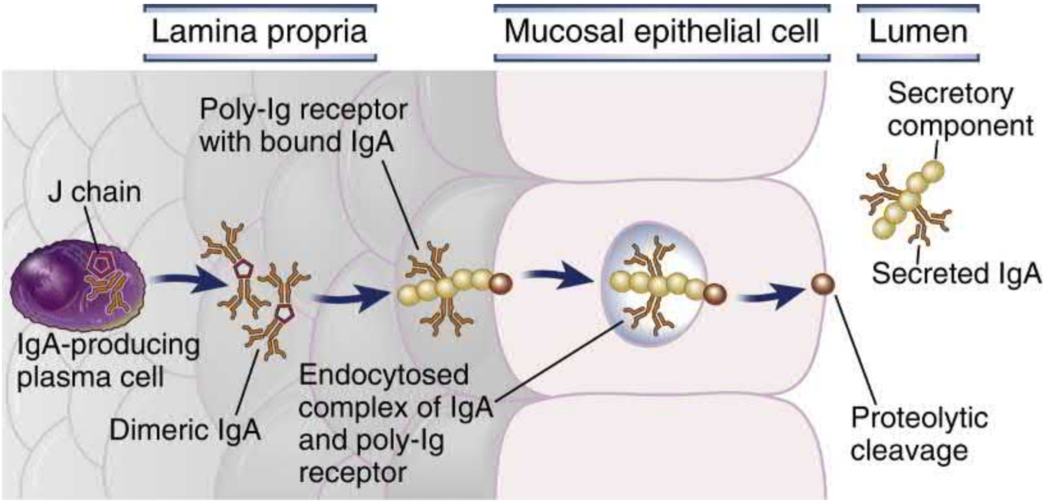

Lamina propria
Mucosal epithelial cell
Lumen
Poly-Ig receptor with bound IgA
J chain
IgA-producing plasma cell
Dimeric IgA
Endocytosed complex of IgA and poly-Ig receptor
Secretory component
Secreted IgA
Proteolytic cleavage

FIGURE 14.6 Transport of IgA across epithelial cells.IgA is produced by plasma cells in the lamina propria of mucosal tissue and binds to the poly-Ig receptor at the base of an epithelial cell. The complex is transported across the epithelial cell, and the bound IgA is released into the lumen by proteolytic cleavage. The process of transport across the cell, from the basolateral to the luminal surface in this case, is called transcytosis.

T cells are found within the gut epithelial layer, scattered throughout the lamina propria and submucosa and around and within follicles in Peyer's patches and other GALT structures. In humans, most of the intraepithelial T cells are CD8$^+$ cells and only about 10% of intraepithelial lymphocytes are γδ cells, which is still higher than the percentages of γδ cells among T cells in other tissues. In mice, about 50% of intraepithelial lymphocytes express the γδ form of the TCR, similar to intraepidermal lymphocytes in the skin. Both the αβ and the γδ TCR–expressing intraepithelial lymphocytes have a limited diversity of antigen receptors, and thus a limited range of specificities compared to most T cells. This restricted repertoire may have evolved to recognize microbes that are commonly encountered at the epithelial surface. Lamina propria T cells are mostly $CD4^+$, and most have the phenotype of previously activated effector or memory T cells (see Chapter 9). Many of the memory T cells are noncirculating tissue-resident memory cells. Recall that these lamina propria effector and memory T cells are generated from naive precursors in the GALT and mesenteric lymph nodes, enter the circulation, and preferentially home back into the lamina propria (see Fig. 14.3). T cells within Peyer's patches and in other follicles adjacent to the intestinal epithelium are mostly $CD4^+$ helper T cells, including follicular helper T cells and regulatory T cells.

DCs and macrophages are abundant in the gastrointestinal immune system and can participate in stimulating protective effector T cell responses or inducing regulatory T cell responses that suppress immunity to ingested antigens and commensal organisms. DCs in the lamina propria take up and process protein antigens from microbes that are in the lumen or have breached the epithelial barrier and transport these antigens via lymphatics to mesenteric lymph nodes (Fig. 14.7). Within the mesenteric lymph nodes, DCs present processed protein antigens to naive T cells and induce the differentiation of these T cells into Th1, Th2, or Th17 effector cells or into $FOXP3^+$ Tregs. Some macrophage-derived DCs in the terminal ileum of the gut project dendrites between epithelial cells and sample luminal contents (see Fig. 14.7). These specialized antigen-sampling cells, identifiable by expression of the chemokine receptor CX3CR, maintain epithelial barrier integrity despite protruding their dendrites between the epithelial cells, by producing the same junctional proteins that the epithelial cells express. These DCs promote protective adaptive immune responses to pathogens in the lumen by passing the sampled antigens to more mobile lamina propria DCs, which then migrate to mesenteric lymph nodes and activate effector T cell responses to those antigens.

In the gastrointestinal tract, different subsets of effector CD4$^+$ T cells are induced by and protect against different microbial species (Fig. 14.8) **.** In Chapter 10, we introduced the concept that helper T cell subsets that secrete different cytokines are specialized for protection against different types of microbes. This fundamental concept is highly relevant to the mucosal immune system. Th1, Th2, and Th17 cells are found in the lamina propria of the intestine, and the commensal bacterial microflora of the gut lumen exerts profound influences on T cell phenotypes, even during homeostasis.

- ***Th17 cells.*** Studies in mice have shown that certain classes of bacteria, or in some cases individual species of bacteria, can shift the dominant pattern of T cell cytokine production. For example, the lamina propria of the small bowel in healthy mice is particularly rich in IL-17–producing cells, whereas the colon is not. The presence of the Th17 cells depends on colonization of the gut with a certain phylum of bacteria (segmented filamentous bacteria) in the postnatal period. These and other bacteria stimulate intestinal epithelial cell production of factors such as serum amyloid A that enhance release of IL-1, IL-6, and TGF-β by DCs, thereby promoting Th17 differentiation. Substances derived from food or intestinal microbes activate the transcription factor aryl hydrocarbon receptor in Th17 cells, which is required for secretion of IL-22. The steady-state presence of Th17 cells is required for protection against pathogenic species of bacteria (e.g., *Citrobacter rodentium*). Th17 cells appear to play a special role in maintaining mucosal epithelial barrier function because of the actions of the two signature cytokines they produce, IL-17 and IL-22, which, as discussed earlier, are also products of the group 3 subset of ILCs in the gut. The receptors for both these cytokines are expressed on intestinal epithelial cells, and both induce the expression of proteins important for barrier function, such as mucins and β-defensins, which protect the epithelial cells against microbe-induced injury.

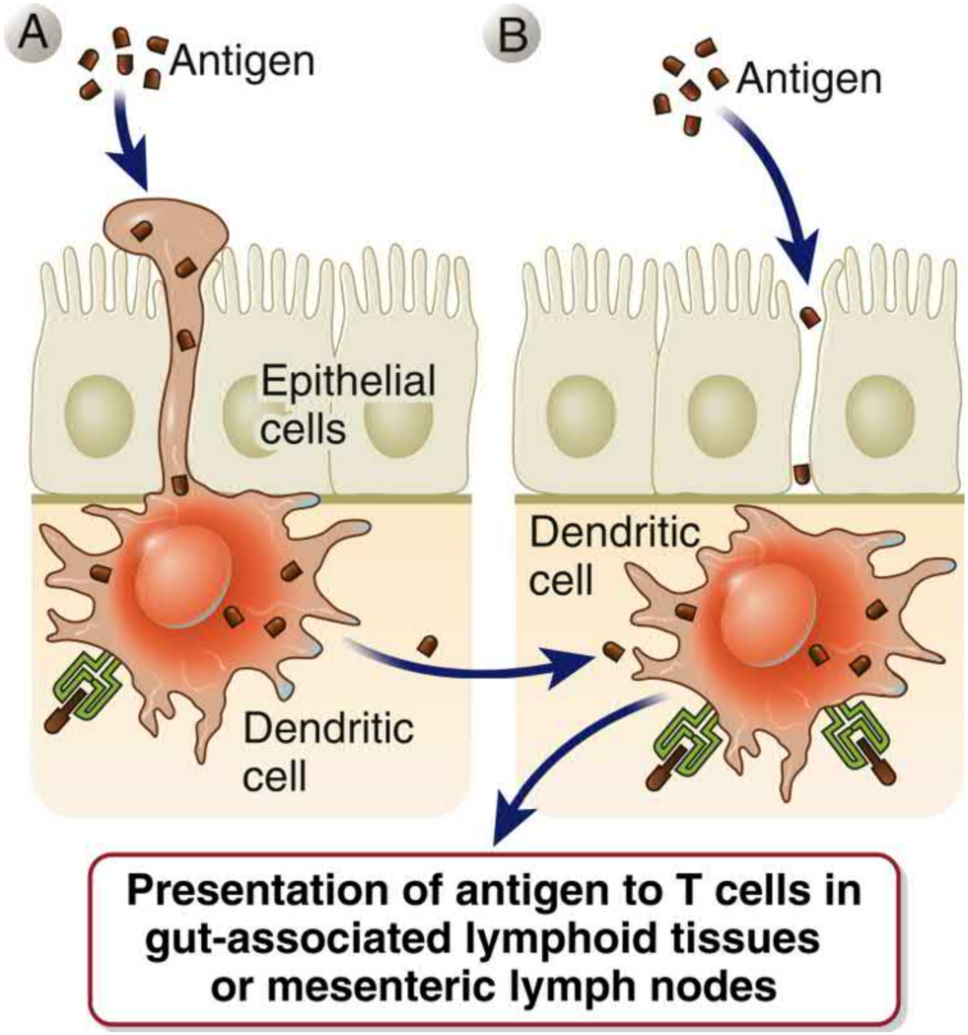

A
Antigen
Epithelial cells
Dendritic cell
B
Antigen
Dendritic cell
Presentation of antigen to T cells in gut-associated lymphoid tissues or mesenteric lymph nodes

FIGURE 14.7 Antigen sampling by intestinal dendritic cells.Dendritic cells *(DCs)* are present in the intestinal mucosa and sample antigens for presentation to T cells in GALT and mesenteric lymph nodes. **A,** Some DCs extend dendritic processes between intestinal epithelial cells into the lumen to sample antigens and then pass the antigens to mobile lamina propria DCs, which migrate into MALT and present the antigens to naive T cells. Macrophages may also sample luminal antigens in this manner. **B,** The lamina propria DCs also sample antigens that are derived from luminal contents and have gotten

through the epithelial barrier and present the antigens to naive T cells in MALT.

- ***Th2 cells.*** Intestinal helminthic infections induce strong Th2 responses, which are effective in eliminating the worms because the Th2 cytokines IL-4 and IL-13 cooperate in enhancing fluid and mucus secretions and inducing smooth muscle contraction and bowel motility.
- ***Th1 cells*** are relatively sparse in healthy lamina propria compared to Th17 or Th2 cells, but their numbers increase in the setting of IBD, and they may contribute to the pathogenesis of this disorder.

Regulation of Immunity in the Gastrointestinal Tract by Regulatory T Cells and Cytokines

Regulatory T cells are abundant in GALT and prevent inflammatory reactions against intestinal commensal microbes. It is estimated that the proportion of FOXP3$^+$ Tregs among CD4$^+$ cells is about twofold greater in the intestine than in other tissues. Many of these Tregs are induced in the gut in response to antigens encountered locally and thus belong to the category of peripheral Tregs (see Chapter 15) (see Fig. 14.8). The factors that contribute to the generation of these peripheral Tregs include local production of retinoic acid and TGF-β by CD103$^+$ DCs and lamina propria macrophages. Both retinoic acid and TGF-β promote FOXP3 expression and inhibit the generation of Th1 and Th2 cells. Furthermore, fermentation metabolites, such as the short-chain fatty acid butyrate produced by intestinal commensal bacteria, especially *Clostridia* species, stimulate peripheral expansion of Tregs. As discussed in Chapter 15, Tregs are thought to suppress immune responses by several mechanisms. Of these, the dominant mechanism in the gut seems to be production of the immunosuppressive cytokine IL-10.

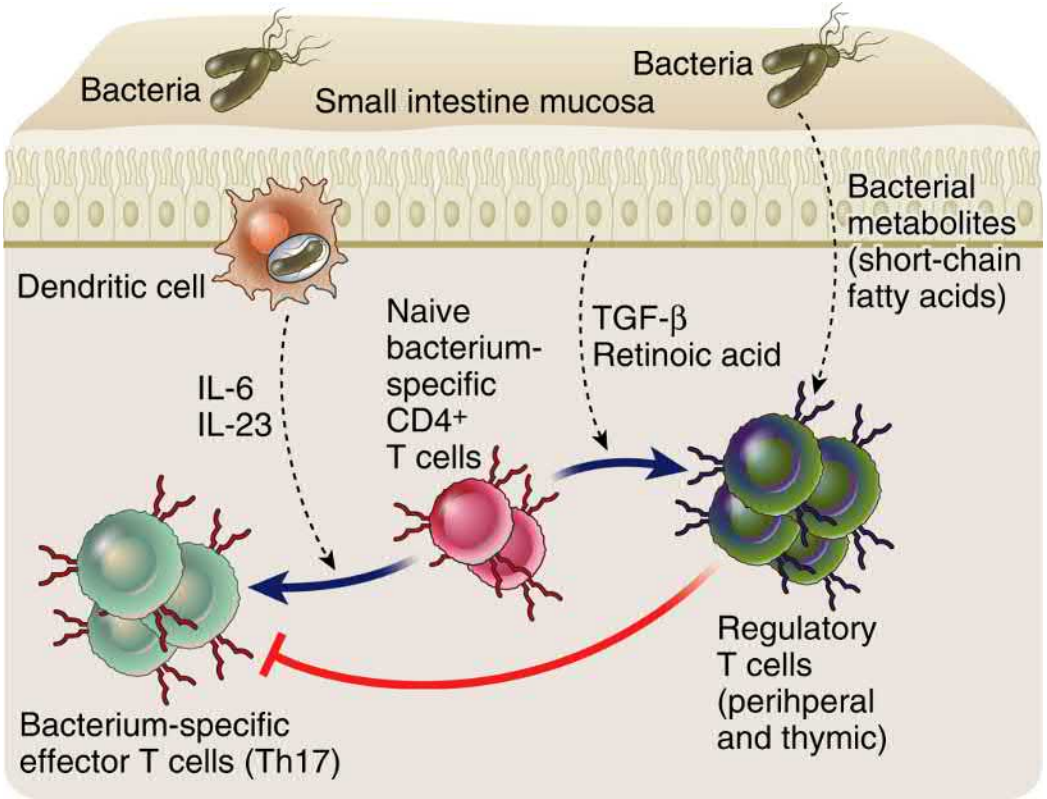

Bacteria
Small intestine mucosa
Bacteria
Bacterial metabolites (short-chain fatty acids)
Dendritic cell
Naive bacterium-specific CD4+ T cells
TGF-β
Retinoic acid
IL-6
IL-23
Regulatory T cells (perihperal and thymic)
Bacterium-specific effector T cells (Th17)

FIGURE 14.8 Effector and regulatory T cells in the intestinal mucosa. Th17 effector T cells and regulatory T cells are abundant in the intestinal mucosa. Bacterial antigen–specific Th17 cells differentiate from naive CD4$^+$ T cells in gut-associated lymphoid tissues (not shown) in response to antigens presented by dendritic cells *(DCs)* and cytokines they secrete, including interleukin-6 *(IL-6)* and *IL-23.* Differentiation of bacterial antigen–specific regulatory T cells (Tregs) is promoted by transforming growth factor-β *(TGF-β)* and retinoic acid produced by intestinal epithelial cells. Thymic Tregs that migrate to the intestine expand in number under the influence of bacterial metabolites. Regulatory T cells require antigen presentation by DCs (not shown); the nature of these antigens is unknown.

Several cytokines, including TGF-β, IL-10, and IL-2, play crucial roles in maintaining homeostasis in the gut immune system, and deficiencies in these cytokines or their receptors result in pathologic bowel inflammation. Much of our knowledge of cytokine-mediated regulation in the gut comes from studies with cytokine or cytokine receptor gene knockout mice. A major feature of the phenotype of mice with engineered deficiencies in TGF-β, IL-10, IL-10 receptor, IL-2, and the IL-2 receptor is uncontrolled inflammation in the bowel. Mutations in the IL-10 receptor gene are also the cause of a rare type of colitis in infants, confirming the importance of IL-10 in preventing pathologic intestinal inflammation in humans. The uncontrolled inflammation observed in the gut in the absence of these cytokines or their receptors is most likely caused by immune responses to commensal gut flora because the inflammation does not occur in mice raised in germ-free conditions.

The cellular sources of the cytokines and the relevant receptor-expressing target cells that are critical for preventing bowel inflammation are not completely defined. Mouse models in which cytokines, cytokine receptors, and cytokine receptor signaling are genetically ablated only in specific cell types have been used to address the question of which cell types are important. In the case of TGF-β–dependent and IL-10–dependent regulation of gut inflammation, evidence indicates that Tregs are an important source of these cytokines. For example, selective deletion of the *Il10* gene in FOXP3$^+$ cells leads to severe colitis, consistent with the critical role of Treg-produced IL-10 in maintaining homeostasis in the gastrointestinal tract. The target cells that express receptors for and are regulated by TGF-β and IL-10 likely include DCs, effector T cells, innate effector cells such as macrophages, and epithelial cells. IBD in mice lacking IL-2 or its receptor is a consequence of defects in the development and function of Tregs, which require IL-2 for their maintenance (see Chapter 15).

Oral Tolerance and Oral Vaccines

Oral tolerance is systemic unresponsiveness to antigens that are ingested or otherwise administered via mucosal tissues. Oral tolerance has been most clearly demonstrated in experimental rodent models. Mice fed high doses of a protein antigen may subsequently show impaired humoral and T cell–mediated responses to the same antigen administered by other routes, such as through the skin. A similar phenomenon can be demonstrated when antigens are administered through the nasal passages into the respiratory mucosa, and the more general term mucosal tolerance is used to describe tolerance induced by oral or nasal antigen administration. The physiologic role of oral tolerance is speculated to be the prevention of potentially harmful immune responses to food proteins and commensal bacteria. The underlying mechanisms of oral tolerance are not well understood but likely include the mechanisms of peripheral tolerance discussed in Chapter 15, such as anergy, deletion, and Treg-mediated suppression. Oral tolerance is systemic because Tregs induced in mucosa may circulate to other tissues, or effector T cells may be killed or rendered unresponsive in the gut and are no longer available to respond to antigens at other sites. Attempts to treat autoimmune disease by oral or nasal administration of relevant self antigens have so far been unsuccessful, but there has been success in reducing development of peanut allergy by oral administration of peanut extract during early childhood (discussed in Chapter 20).

Oral administration of antigen in the setting of concomitant stimulation of innate immunity can lead to productive adaptive immune responses, as in the use of oral vaccines to induce protective antibody responses to poliovirus or the bacterium S. typhi. These vaccines are live attenuated microbes that may infect cells in the intestine and stimulate strong innate responses that then promote T and B cell activation.

The Role of the Commensal Microbiome in Immune Regulation

The human intestinal microbiome includes all of the commensal bacteria that normally reside in the intestines, discussed earlier, as well as thousands of species of viruses, fungi, and protozoans. Humans and their intestinal microbiome have co-evolved mechanisms for mutual benefit, including mechanisms to defend against invasion by these organisms together with mechanisms to maintain equilibrium by minimizing unneeded proinflammatory immune responses to the commensal organisms. One consequence of this co-evolution is a profound influence of the microbiome on the immune system. The microbiome changes with age, diet, and disease, and experimental studies indicate that these changes affect immune function locally in the gut and systemically.

Commensal organisms in the intestines induce and regulate innate immune responses in the gut and also influence systemic innate immunity. Studies in mice have shown that commensal bacteria are needed for proliferation and repair of the intestinal epithelial barrier after injury, an effect mediated by bacterial cell wall PAMPs and the TLRs to which they bind on the epithelial cells. As mentioned earlier, microflora in the gut stimulate the expression of mucins and antimicrobial molecules (including defensins and REGIIIγ) that prevent bacterial colonization. In addition, several studies in mice have shown that products of commensal bacteria in the gut influence the way circulating neutrophils and macrophages function systemically. For example, short-chain fatty acids from gut bacteria dampen neutrophil inflammatory responses, whereas fragments of intestinal bacterial peptidoglycan enhance the ability of circulating neutrophils to kill gram-positive bacteria. Likewise, gut bacteria appear to be required for systemic antiviral functions of macrophages, DCs, and natural killer (NK) cells.

Intestinal commensal organisms influence local and systemic adaptive immune responses. In mice, the production of IgA in the intestinal mucosa, which is a major adaptive immune mechanism for protection against microbial invasion through the intestinal epithelial barrier, is dependent on the presence of a subset of small bowel luminal bacterial flora. Commensal bacterial antigens activate specific IgA responses by inducing the expression of BAFF, APRIL, and retinoic acid, which are IgA switch factors required for T-dependent and T-independent B cell class switching to IgA (discussed earlier). By preventing commensals reaching the barrier epithelium, IgA in the gut reduces innate responses to these organisms, and limits B cell activation and antibody responses locally and systemically. Certain species of commensal organisms are also required for accumulation of Th17 cells in the gut, as discussed earlier, and the presence of these species reduces resistance to some gut pathogens but may increase susceptibility to autoimmune disease outside the gut. Other commensal species contribute to the development of Tregs.

In humans, the impact of gut microflora on local and systemic immune responses is inferred from many clinical observations and experimental therapies. Normal flora appears to be required to prevent harmful intestinal innate responses and inflammation induced by pathogenic bacteria. For example, antibiotic treatment for infections outside the gut will invariably alter the makeup of the gut microflora, and this is associated with increased risk for pathologic bacterial infections in the colon, especially with *Clostridium difficile*. Patients with chronic *C. difficile* infection benefit from orally administered fecal transplants, which repopulate the gut with flora from healthy individuals.

It has been suggested that alterations in the intestinal microbiome, which is sometimes called dysbiosis, may underlie the development of numerous disorders, but how human commensal gut flora influence systemic immunologic health is largely unknown. The risk for developing allergic disease, including asthma, has been linked to variations in microflora acquired during early childhood as a consequence of mode of birth (vaginal versus cesarean section), breast-feeding, and antibiotic use. Currently, the microbiomes of various normal and patient populations are being characterized by genetic methods. Although this work may lead to a better understanding of how the human immune system is regulated by gut bacteria, a major challenge in interpreting the data is the significant variation of the human microbiome among individuals and over time even in one person.

Immunity in Other Mucosal Tissues

Like the gastrointestinal mucosa, the mucosae of the respiratory system and the genitourinary system must maintain a barrier against invasion of diverse microbes in the environment and balance effective protective responses to invading microbes and suppression of responses to commensal organisms. Many of the features we described for gastrointestinal immunity are shared by mucosal immunity in these different locations. These shared features include relatively impermeable mucus- and defensin-secreting epithelial barriers; localized collections of lymphoid tissues beneath the epithelium; the constant sampling of antigens located outside the barriers by immune cells within the barrier; the integration of proinflammatory and regulatory signals generated by microbial products binding to epithelial and DC innate immune receptors; the strong reliance on secretory IgA–mediated humoral immunity to prevent microbial invasion; and the presence of DC populations that stimulate particular types of effector and regulatory T cell responses. In addition to these shared features, different mucosal tissues have unique properties that reflect the distinct functions and anatomy of the organs of which it is part and the range of environmental antigens and microbes that are present at each site. We will now discuss some of the major features of mucosal immunity in the respiratory and genitourinary systems.

Immunity in the Respiratory System

The mucosa of the respiratory system lines the nasal passages, nasopharynx, trachea, and bronchial tree. Alveoli, the epithelium-lined sac-like termini of the bronchial airways, also may be considered part of the respiratory mucosa. Inhalation of air exposes the respiratory mucosa to a wide variety of foreign substances, including airborne infectious organisms, plant pollens, dust particles, and various other environmental antigens. The microbial flora of the airways is less dense and less diverse than that in the gut, and the deep airways and alveoli have fewer organisms than the upper airways. Nonetheless, similar mechanisms have evolved in the respiratory mucosal immune system to achieve a balance between immune activation to protect against pathogens and immune regulation to avoid unnecessary or excessive responses that might impair physiologic functions. Failure of the immune system to control bronchopulmonary infections and excessive immune or inflammatory responses to infections are major causes of morbidity and mortality worldwide.

Innate Immunity in the Respiratory System

The pseudostratified, ciliated columnar epithelium that lines most of the respiratory mucosa, including the nasal passages, nasopharynx, and bronchial tree, performs physical and chemical barrier functions similar to those of gut epithelium, by virtue of tight junctions between cells and secretion of mucus, defensins, and cathelicidins. The mucus in the airways traps foreign substances, including microbes, and the cilia move the mucus and trapped microbes up and out of the lungs. The importance of mucus and cilia in innate immune protection in the lung is illustrated by the greatly increased frequency of serious bronchopulmonary infections in people with decreased cilia function, such as heavy smokers, or abnormal mucus production, such as patients with cystic fibrosis.

Innate responses in alveoli serve antimicrobial functions but are tightly controlled to prevent inflammation, which would impair gas exchange. The alveoli are susceptible to infection spreading from bronchopneumonia, and alveolar lining cells can be directly infected by viruses. Surfactant proteins A (SP-A) and D (SP-D), which are secreted into the alveolar spaces, are members of the collectin family (see Chapter 4) and bind to carbohydrate PAMPs on the surface of many pathogens. These surfactants are involved in viral neutralization and clearance of microbes from the alveoli, but they also suppress inflammatory and allergic responses in the lung. For example, SP-A inhibits TLR2 and TLR4 signaling and the production of inflammatory cytokines in alveolar macrophages, and

SP-A also binds to TLR4 and inhibits lipopolysaccharide binding. SP-A and SP-D reduce the phagocytic activity of alveolar macrophages.

Alveolar macrophages represent most of the free cells within the alveolar spaces. These tissue-resident macrophages are functionally distinct from those in most other tissues in that they maintain an antiinflammatory phenotype. They express IL-10, nitric oxide, and TGF-β and are poorly phagocytic compared with resident macrophages in other tissues, such as the spleen and liver. Alveolar macrophages inhibit T cell responses as well as the antigen presentation function of airway DCs, effects that are attributed to the IL-10 and TGF-β they secrete.

ILCs are present in bronchial mucosal tissue, and contribute to pulmonary inflammatory responses to helminths and environmental allergens. Damage to bronchial epithelium by microbes results in the release of alarmin cytokines such as IL-33, which activate ILC2s and Th2 cells. Pulmonary ILC2s also can be activated by neuropeptides derived from autonomic nerves and also from bronchial neuroendocrine cells.

Adaptive Immunity in the Respiratory System

Protective humoral immunity in the airways is dominated by secretory IgA, as in other mucosal tissues, although the amount of IgA secreted is less than in the gastrointestinal tract. Secretory IgA plays an important role in the upper airway. The anatomic sites of naive B cell activation, differentiation, and IgA class switching include tonsils and adenoids in the nasopharynx and lymph nodes in the mediastinum and adjacent to bronchi in the lungs. There are relatively few aggregated or isolated lymphoid follicles in the lamina propria in the lower airways compared with the gut and likely less initiation of humoral immune responses in these locations. The homing of IgA-secreting plasmablasts back into the airway tissue in proximity to respiratory mucosal epithelium depends on the chemokine CCL28 secreted by respiratory epithelium and its receptor CCR10 on the plasma cells. IgA is transported into the airway lumen by the same poly-Ig receptor mechanism of transcellular transport as in the gut. IgE responses to airway antigens occur frequently and are involved in allergic diseases of the respiratory system, including hay fever and asthma. IgE performs its inflammatory effector functions when bound to mast cells, which are abundant in the submucosa of the airways.

T cell responses in the lung are initiated by DC sampling of airway antigens and presentation of these antigens to naive T cells in peribronchial and mediastinal lymph nodes. A network of DCs is present in the mucosa of the airways, and a subset of these bronchial DCs extend dendrites between the bronchial epithelial cells into the airway lumen. These DCs sample airway antigens, migrate to draining lymph nodes, and present the processed antigens to naive T cells. Other DCs are found in the lamina propria beneath the epithelial cells, where they capture antigens that cross the epithelium. Airway DCs may drive differentiation of naive $CD4^+$ T cells toward different phenotypes depending on the nature of the antigen and the disease context. For example, Th1 cells are often generated in viral infections, while Th2 cells are frequent in allergic responses to inhaled allergens in asthma.

Immunity in the Genitourinary System

Innate immune defense against microbial invasion and infection in the genitourinary mucosa relies mainly on the epithelial lining, as in other mucosal barriers. Stratified squamous epithelium lines the vaginal mucosa and terminal male urethra, and a single layer of mucus-secreting columnar epithelium lines the female genital tract. The vaginal epithelium contains Langerhans cells, and a variety of DCs and macrophages have been described beneath the epithelium in the vagina, endocervix, and urethra. There are also resident B and T cells in the genital mucosa. The adaptive immune system in the genitourinary mucosa lacks prominent MALTs. Unlike other mucosa, in which IgA is the dominant antibody isotype, most of the antibody in genital secretions is IgG, about half of which is produced by plasma cells in genital tract mucosa and the rest is from the circulation. Infections in the genitourinary tract are a major cause of morbidity and mortality worldwide and occur when the epithelial barriers are damaged, urinary tract function is impaired, or microbes evade the local mechanisms of defense.

The Cutaneous Immune System

The skin includes two main layers, the outer epidermis, composed mainly of epithelial cells, and, separated by a thin basement membrane, the underlying dermis composed of connective tissue and specialized adnexal structures such as hair follicles and sweat glands. Within both of these layers, there are a variety of different cell types and their products, comprising the cutaneous immune system, which provide physical barrier and active immune defense functions against microbes (Fig. 14.9). The skin of an adult is about 2 m^2 in area and is the second-largest barrier of the body against environmental microbes and other foreign materials. Given its outermost location, the skin is normally colonized by many microbes and is frequently breached by trauma and burns. Therefore, the skin is a common portal of entry for a wide variety of microbes and other foreign substances and is the site of many immune responses.

Innate and Adaptive Immune Responses in the Skin

The epidermis provides a physical barrier to microbial invasion. The epidermis consists of multiple layers of stratified squamous epithelium, made up almost entirely of specialized epithelial cells called keratinocytes. The basal layer of keratinocytes, anchored onto the basement membrane, continuously proliferate, and their maturing progeny cells are displaced upward and differentiate to form several different layers. In the top layer, called the stratum corneum, the cells undergo programmed death, thereby forming a keratin- and lipid-rich permeability barrier that is important for protection against microbes and harmful physical and chemical agents.

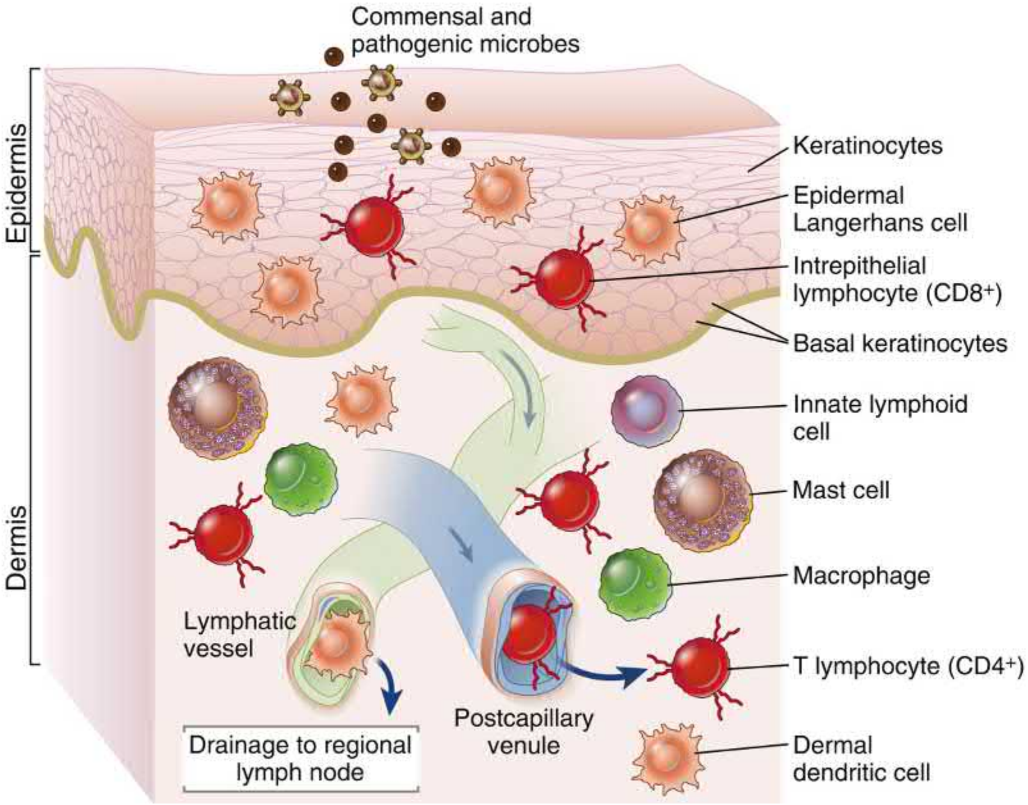

Commensal and pathogenic microbes
Epidermis
Dermis
Keratinocytes
Epidermal Langerhans cell
Intrepithelial lymphocyte (CD8+)
Basal keratinocytes
Innate lymphoid cell
Mast cell
Macrophage
T lymphocyte (CD4+)
Dermal dendritic cell
Lymphatic vessel
Drainage to regional lymph node
Postcapillary venule

FIGURE 14.9 Cellular components of the cutaneous immune system.The major components of the cutaneous immune system shown in this schematic diagram include keratinocytes, Langerhans cells, and intraepithelial lymphocytes, all located in the epidermis, and T lymphocytes, innate lymphoid cells, dendritic cells, and macrophages, located in the dermis.

In addition to forming a physical barrier, keratinocytes actively respond to pathogens and injury by producing antimicrobial peptides, which kill microbes, and various cytokines, which promote and regulate immune responses. The antimicrobial peptides that keratinocytes produce include defensins, S100, and cathelicidins (see Chapter 4). The cytokines made by keratinocytes include TNF, TSLP, IL-1, IL-6, IL-18, IL-25, and IL-33, which promote inflammation; granulocyte-macrophage colony-stimulating factor (GM-CSF), which induces differentiation and activation of DCs in the epidermis (discussed later); and IL-10, which controls immune responses. Keratinocytes produce the chemokine CCL27, which participates in recruitment of lymphocytes expressing CCR10. The expression of defensins, cytokines, and chemokines by keratinocytes is induced by innate immune receptors, including TLRs and NLRs (see Chapter 4). Keratinocytes in normal skin constitutively synthesize pro–IL-1β and pro–IL-18. Stimuli such as ultraviolet irradiation activate the inflammasome to process these pro-cytokines to the active forms, which explains the inflammatory response to sunburn. When signal transduction pathways linked to inflammatory responses, such as the NF-κB (nuclear factor κB) and STAT3 (signal transducer and activator of transcription3) pathways, are genetically activated only in keratinocytes, mice develop inflammatory skin diseases, showing the potential of keratinocytes to act as central players in cutaneous immune responses.

Innate immune responses to pathogens that breach the epidermal barrier are initiated by macrophages, mast cells, and ILCs in the dermis. As we have described for other tissues, tissue-resident sentinel cells such as macrophages and mast cells express TLRs and other innate pattern recognition receptors and respond to PAMPs and DAMPs (damage-associated molecular patterns) by secreting inflammatory cytokines and lipid mediators. ILCs are activated by cytokines secreted by keratinocytes and sentinel cells and in turn secrete inflammatory cytokines, which influence the type of inflammatory responses that follow. For example, ILC2s are activated by keratinocyte-derived TSLP, IL-25, and IL-33, and the ILC2s then secrete IL-5, which promotes eosinophilic inflammation. IL-18 production by keratinocyte and sentinel cells activates ILC1s to secrete IFN-γ, which promotes macrophage-mediated defense. DCs also play an important sentinel role in the skin, as is discussed in more detail next.

Several DC populations are normally present in the skin and contribute to innate immune responses and initiation of T cell

responses to microbial and environmental antigens that enter the body through the skin. In the epidermis, the most abundant DCs are Langerhans cells (see Fig. 2.5), which express a C-type lectin receptor called langerin (CD207) and have numerous Birbeck granules in the cytoplasm. Langerhans cells populate the skin during embryonic development and are developmentally related to other tissue-resident macrophages rather than conventional DCs. The dendrites of Langerhans cells form a dense meshwork between the keratinocytes of the epidermis. In the dermis, there are relatively sparse langerin-expressing DCs, which express CD103 in mice and CD141 in humans and are a distinct lineage from Langerhans cells. Each of these DC populations expresses innate pattern recognition receptors for PAMPs as well as for DAMPs derived from injured cells. The DCs respond to these ligands by secreting inflammatory cytokines.

Both epidermal Langerhans cells and dermal DCs take up protein antigens, process them into peptides, and migrate to draining lymph nodes where they present peptide-MHC complexes to naive T cells (see Chapter 6). The contributions of the different skin DC subsets to the initiation of different types of T cell responses are not fully defined. Mouse models have been developed in which particular DC subsets are eliminated, and these models show that mouse Langerhans cells are not required for activation of $CD4^+$ and $CD8^+$ T cell responses to many types of antigens in the skin, but they do appear to play a role in Th17 responses to extracellular pathogens, and in tolerance to some skin antigens. Langerin-expressing cDC1s in mice and humans are required for cross-presentation of antigens to naive $CD8^+$ T cells.

Normal human skin contains many T cells, 95% of which have a memory phenotype. There are about 2×10^{10} total T cells in the skin. About 98% of these T cells are present in the dermis, and 2% are intraepidermal lymphocytes. Dermal T lymphocytes (both $CD4^+$ and $CD8^+$ cells) are located predominantly adjacent to blood vessels and hair follicles. Most of these dermal T cells are tissue-resident memory cells generated within lymph nodes during prior skin infections, which then home to and remain in the skin for long periods without recirculating. Smaller numbers of both $CD4^+$ and $CD8^+$ resident memory T cells are present in the epidermis and express the integrin CD103, which binds to ligands on epithelial cells and retains the T cells in the skin. All of these resident memory T cells display potent effector functions when activated by antigen and include $CD4^+$ cells of each major helper subset, Th1, Th2, Th17, and Treg. Th1 and Th17 cells are important for microbial defense against intracellular and extracellular microbes, respectively, as in other tissues. The two signature Th17 cytokines, IL-17 and IL-22, induce expression of defensins and cathelicidins by keratinocytes and IL-22 induces epidermal cell proliferation. In contrast, the Th2 cytokines IL-4 and IL-13 suppress production of defensins and cathelicidin, which can result in infections in Th2-driven skin diseases. Dermal γδ T cells may be a source of IL-17 in some chronic inflammatory skin diseases.

T cells in the skin express homing molecules that direct their migration out of dermal microvessels (Fig. 14.10) **.** Migration of effector or memory T cells into the skin depends on T cell expression of cutaneous lymphocyte antigen (CLA), which is an E-selectin–binding carbohydrate moiety displayed on various glycoproteins on the endothelial cell plasma membrane. In addition, T cell expression of CCR4, CCR8, and CCR10, which bind the chemokines CCL17, CCL1, and CCL27, respectively, is also required for T cell trafficking to skin. The skin-homing properties of T cells are imprinted during activation in skin-draining lymph nodes, by a process analogous to imprinting of gut-homing properties of T cells in mesenteric lymph nodes, discussed earlier in the chapter. When naive T cells recognize antigens presented by DCs in skin-draining lymph nodes, the T cells receive signals from the DCs that not only induce proliferation and differentiation into effector cells but also induce expression of the skin-homing molecules CLA, CCR4, CCR8, and CCR10. Interestingly, sunlight and vitamin D appear to play an important role in T cell migration to the skin, analogous to the role of vitamin A and its metabolite retinoic acid in lymphocyte migration to the gut. UVB rays in sunlight act on 7-dehydrocholesterol made in the basal layer of the epidermis, converting it to previtamin D_3. Dermal DCs express vitamin D_3 hydroxylases that convert previtamin D_3 to the active form, $1,25(OH)_2D_3$, which may be transported in free form or within migrating DCs to skin-draining lymph nodes. Within the node, $1,25(OH)_2D_3$ enters T cells that have been activated by antigen-presenting DCs, translocates to the nucleus, and induces transcription of CCR10. IL-12 made by the DCs participates in induction of CLA. CCR4 and CCR8 are also upregulated, and the gut-homing integrin $\alpha_4\beta_7$ is downregulated, by unknown signals, during T cell activation in skin-draining lymph nodes. Thus, naive T cells activated in skin-draining lymph nodes will differentiate into effector T cells that preferentially home back into the skin. $1,25(OH)_2D_3$ may also act locally within the dermis on effector and memory T cells to upregulate CCR10 and promote migration of the T cells into the epidermis in response to the CCR10 ligand CCL27 made by keratinocytes.

Immune-Privileged Tissues

Immune responses and associated inflammation in certain parts of the body, including brain, eye, testes, placenta, and fetus, carry a high risk for lethal organ dysfunction or reproductive failure. These tissues, which have evolved to be protected, to a variable degree, from immune responses, are called **immune-privileged sites.** Peter Medawar coined the term immune privilege in the 1940s to describe the lack of immune responses to tissue transplanted into the brain or the anterior chamber of the eye of experimental animals. Foreign antigens that would evoke an immune response in most tissues are often tolerated in these immune-privileged sites. The mechanisms underlying immune privilege vary between these tissues and are not fully understood. Some of the mechanisms are similar to mechanisms of regulation in gut and skin (discussed earlier) and mechanisms of self-tolerance (discussed in Chapter 15).

Immune Privilege in the Brain, Eye, and Testis

The Brain

Inflammation in the brain can lead to functional derangement and death of neurons, with disastrous consequences. A major feature of the brain that impairs inflammatory responses is the presence of vascular barriers that impede movement of inflammatory cells and microbes into the brain. If these barriers are broken, common pathogens may stimulate strong inflammatory responses that damage the brain parenchyma. The main vascular barrier is the blood-brain barrier, which includes the endothelial layer of small vessels, with tight junctions that are less leaky than in other vascular beds. In addition, pericytes, which are contractile cells that surround the endothelial layer, are also key to limiting the movement of blood leukocytes and microbes out of cerebral vessels. Furthermore, the cerebral vessel pericytes have been found to inhibit T cell responses in part by producing TGF-β and retinoic acid and inducing regulatory T cell differentiation. Perivascular macrophages and glial cells provide additional blood-brain barrier functions against pathogen entry and immune cell trafficking, and even if T cells have migrated out of vessels, they are often found restricted to a cuff around the vessel and do not readily migrate deep into the brain parenchyma. In addition, there are pial and epithelial barriers that under normal conditions impede the movement of leukocytes and microbes from blood into the cerebrospinal fluid (CSF) and from the CSF into the brain parenchyma and separate the CSF from the brain parenchyma. Nonetheless, when pathogens do gain access to the CSF, there can be robust inflammatory responses, such as occurs in bacterial meningitis.

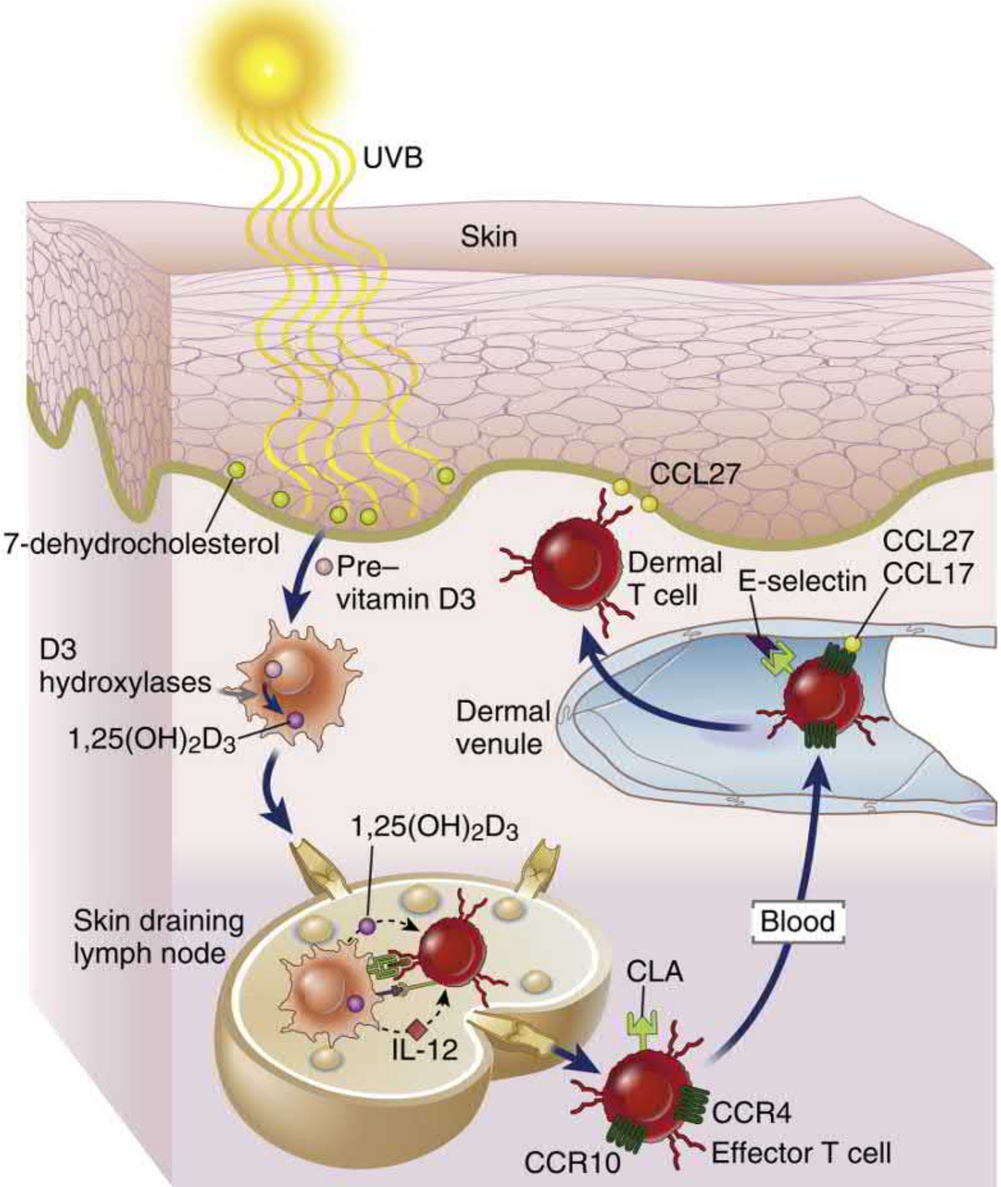

UVB
Skin
7-dehydrocholesterol
Pre–vitamin D3
D3 hydroxylases
$1,25(OH)_2D_3$
CCL27
Dermal T cell
E-selectin
CCL27
CCL17
Dermal venule
$1,25(OH)_2D_3$
Skin draining lymph node
IL-12
Blood
CLA
CCR4
Effector T cell
CCR10

FIGURE 14.10 Homing properties of skin lymphocytes. The skin-homing properties of effector lymphocytes are imprinted in skin-draining lymph nodes, where they have undergone differentiation from naive precursors. Ultraviolet rays in sunlight *(UVB)* stimulate production of vitamin D, which induces expression of CCR10; interleukin-12 *(IL-12)* induces expression of the E-selectin ligand CLA; and other signals induce CCR4, CCR8, and CCR10 expression. These homing molecules direct migration of the effector T cells into the skin. *CLA,* Cutaneous lymphocyte antigen.

Besides these barriers that limit access of the brain to infectious organisms and to immune cells, strong innate responses and initiation of adaptive immunity to antigens in the brain may be relatively impaired compared to other tissues by other mechanisms. These include inhibitory signaling pathways that block activation of microglial cells, which are the major tissue-resident macrophages in the brain, and a relative scarcity of DCs. The initiation of a T cell response to a microbe in the brain would most likely require delivery of the antigen into CSF and then to meningeal lymphatic vessels that drain to cervical lymph nodes.

Contrary to common assumptions about immune privilege, there is evidence that immune surveillance against microbes does occur in the central nervous system (CNS). For example, the frequency of some opportunistic infections within the brain increases significantly in immunosuppressed patients. One example is the activation of latent JC virus in patients with primary, acquired, and drug-induced immunosuppression, leading to a uniformly fatal CNS disease called progressive multifocal leukoencephalopathy (PML). One of the drugs that is associated with increased risk for PML is a monoclonal antibody that blocks integrin-mediated monocyte and T cell recruitment into the brain, which is used to treat patients with multiple sclerosis and IBD. This observation suggests that T cell or monocyte trafficking into the brain is necessary to keep latent viruses in check and argues that the brain is not a stringently immune-privileged site.

The Eye

Vision, which is essential for the survival of most vertebrates, can be easily impaired by inflammation within the eye. Various anatomic and physiologic features have evolved that minimize the likelihood of immune responses and inflammation in the eye. Many of these features are analogous to those that underlie immune privilege in the brain. The retina is anatomically an extension of the CNS and is protected by a blood-retinal barrier with many of the same vascular wall properties as the blood-brain barrier. The anterior chamber of the eye is the fluid-filled space between the transparent cornea in front and the iris and lens behind. Inflammation in this chamber could lead to opacification of the transparent cornea and lens, with loss of sight. The fluid in this space, aqueous humour, is biochemically similar to CSF, and in terms of access to the immune system, is analogous to lymph, in that it drains into veins or into conjunctival lymphatics. Despite this access, the anterior chamber of the normal eye maintains a significant degree of immune privilege. Anatomic features of the anterior chamber that contribute to immune privilege include the tight junctions of the epithelial layer and resistance to leakiness of blood vessels in the tissues adjacent to the anterior chamber, and the avascular nature of the cornea. There are several soluble factors with immunosuppressive and antiinflammatory properties in the aqueous humor that fills the anterior chamber, including neuropeptides (α-melanocyte–stimulating hormone, vasointestinal peptide, somatostatin), TGF-β, and indolamine 2,3-dioxygenase (IDO, discussed later). Cells lining the anterior chamber, including the epithelium of the iris and the endothelium, constitutively express FAS ligand and PD-L1, which can induce death or inactivation of T cells, respectively.

One liability of the sequestration of self antigens in the eye is that immune tolerance to these antigens is not induced. This lack of tolerance becomes a problem only when trauma exposes the eye antigens to the immune system. A striking example of this is sympathetic ophthalmia, in which trauma to one eye causes release of eye antigens leading to autoimmune disease in both the injured eye and the uninjured eye. Presumably, although self antigens in the normal eye are inaccessible to the extraocular immune system to induce either autoimmunity or tolerance, activated immune effector cells and antibodies that are generated in the periphery when one eye is injured have access to and cause injury to the normal eye.

An experimental phenomenon called anterior chamber–associated immune deviation occurs when an antigen placed in the anterior chamber of a mouse eye results in systemic immune tolerance to that antigen. A similar phenomenon has been described when the antigen is placed in the brain. Several different mechanisms have been proposed, but the relevance of this phenomenon to immune privilege is unclear.

The Testis

Immune privilege in the testis serves to limit inflammation that may impair male fertility. Many self antigens in the adult testis are first expressed at the time of puberty, well after the development of a competent immune system, so it is unlikely that lymphocytes specific for these antigens are deleted during development. Therefore, immune privilege in the testis may also serve to prevent autoimmunity. The testis, like the eye and brain, has a blood-tissue barrier that limits delivery of cells and molecules to the sites of spermatogenesis. This barrier is not formed by endothelial cells but rather by Sertoli cells, which line the outer layer of the seminiferous tubules where spermatogenesis takes place. The hormonal milieu of the testis, which is rich in androgens, has an antiinflammatory influence on macrophages. TGF-β is produced by Leydig, Sertoli, and peritubular cells and likely contributes to local immune suppression.

Immunity in the Mammalian Fetus and Newborn

Immune Privilege of the Mammalian Fetus

In eutherian mammals (mammals with placentae), the fetus expresses paternally inherited genes that are foreign to the mother, but fetuses are not normally rejected by the mother. In essence, the fetus is a naturally occurring allograft, but one that is protected from graft rejection. (Allograft rejection is discussed in Chapter 17.) It is clear that the mother is exposed to fetal antigens during pregnancy because maternal antibodies against paternal major histocompatibility complex (MHC) molecules are easily detectable. Obviously, there has been very strong selective pressure that has led to the evolution of mechanisms that protect the fetus from the maternal immune system, so called fetal tolerance, yet these mechanisms remain poorly understood. Many different special molecular and barrier features of the placenta and local immunosuppression may contribute.

The anatomic location of the fetus is a critical factor in the absence of rejection. For example, pregnant animals are able to recognize and reject allografts syngeneic to the fetus placed at extrauterine sites without compromising fetal survival. Wholly allogeneic fetal blastocysts that lack maternal genes can successfully develop in a pregnant or pseudopregnant mother. Thus, neither specific maternal nor paternal genes are necessary for survival of the fetus. Hyperimmunization of the mother with cells bearing paternal antigens does not compromise placental and fetal growth.

The failure to reject the fetus has focused attention on the region of physical contact between the mother and fetus. The fetal tissues of the placenta that most intimately contact the mother are composed of vascular trophoblast, which is exposed to maternal blood for purposes of mediating gas exchange and nutrient supply, or implantation site trophoblast, which diffusely infiltrates the uterine lining (decidua) for purposes of anchoring the placenta to the mother.

One simple explanation for fetal survival is that trophoblast cells fail to express paternal MHC molecules. Class II MHC molecules have not been detected on trophoblast cells. In mice, cells of implantation trophoblast, but not of vascular trophoblast, do express paternal class I MHC molecules. In humans, the situation may be more complex in that trophoblast cells express mainly a nonpolymorphic class I molecule called HLA-G. This molecule may be involved in protecting trophoblast cells from maternal NK cell–

mediated lysis. A specialized subset of NK cells called uterine NK cells are the major type of lymphocyte present at implantation sites, and interferon-γ (IFN-γ) production by these cells is essential for decidual development. The way in which uterine NK cells are stimulated and their role in maternal responses to fetal alloantigens are not known. Even if trophoblast cells do express classical MHC molecules, they may lack costimulator molecules and fail to act as antigen-presenting cells.

The uterine decidua may be a site where immune responses are functionally inhibited. In support of this idea is the observation that mouse decidua is highly susceptible to infection by *Listeria monocytogenes* and cannot support a delayed-type hypersensitivity response. The basis of immunologic privilege is clearly not a simple anatomic barrier because maternal blood is in extensive contact with trophoblast cells. Rather, the immune barrier is likely to be created by functional inhibition, attributable to multiple mechanisms.

Maternal tolerance of the fetus may be mediated by Tregs. Experimental evidence suggests that Treg cells prevent immune reactions against paternally derived antigens that are not expressed in the mother. Fetal antigens induce long-lived FOXP3$^+$ Tregs in mice, and depletion of these cells results in fetal loss. During pregnancy, systemic and decidual Tregs increase in mothers and abundant Tregs are found in the fetus. Indeed, eutherian mammals have evolved a transposon-mediated change in a regulatory sequence of the *Foxp3* gene that allows these species to generate stable peripheral Treg. This regulatory region of *Foxp3* is not found in earlier vertebrates or even in metatherian mammals such as kangaroos and wallabies that carry their young. The contribution of Tregs in human pregnancy is under active investigation, as is the possibility of Treg defects as the basis for recurrent spontaneous abortions.

Immune responses to the fetus may be regulated by local concentrations of tryptophan and its metabolites in the decidua, which inhibit T cell responses. The enzyme IDO is made in the placenta and catabolizes tryptophan, generating a by-product, kynurenine. Tryptophan is required for proliferating cells, including lymphocytes, and kynurenine is toxic to these cells. These observations led to the hypothesis that T cell responses to the fetus are normally blocked because decidual tryptophan levels are kept low or the levels of toxic metabolites produced by IDO are high.

Several other mechanisms may also dampen the maternal immune response to the fetus, including FASL expression by fetal trophoblast cells that promote apoptosis of activated FAS-expressing maternal lymphocytes, and the induction by galectin-1 in the decidua of tolerogenic DCs that facilitate Treg generation.

Passive Immunity in the Fetus and Newborn

Fetal and neonatal mammals cannot make their own IgG antibodies, but they defend against infection by maternal IgG transported across the placenta into the fetal circulation and by IgA in breast milk ingested by the nursing infant. Transport of maternal IgG across the placenta is mediated by the IgG-specific **neonatal Fc receptor (FcRn)**. This receptor is expressed on syncytiotrophoblast cells of the placental villi, which form a barrier between the maternal and fetal tissues. FcRn binds IgG from the maternal blood present in the intervillous spaces, and the receptor mediates transcytosis of the IgG to the fetal side of the syncytiotrophoblast, where it is released into the villous stroma. The IgG is then transported across fetal capillary endothelium into the fetal circulation (Fig. 14.11A). Thus, a newborn contains essentially the same IgG antibodies as the mother. Protective levels of maternal IgG are maintained for about 6 months, after which time the majority of circulating IgG is made by the baby (Fig. 14.11B). This transition period is a time of enhanced susceptibility of babies to infections because they will not have accumulated memory B cells and long-lived plasma cells from prior infections. The transepithelial transport of maternal IgA into breast milk depends on the poly-Ig receptor described earlier this chapter, and the ingested IgA can neutralize pathogenic organisms that attempt to colonize the infant's gut.

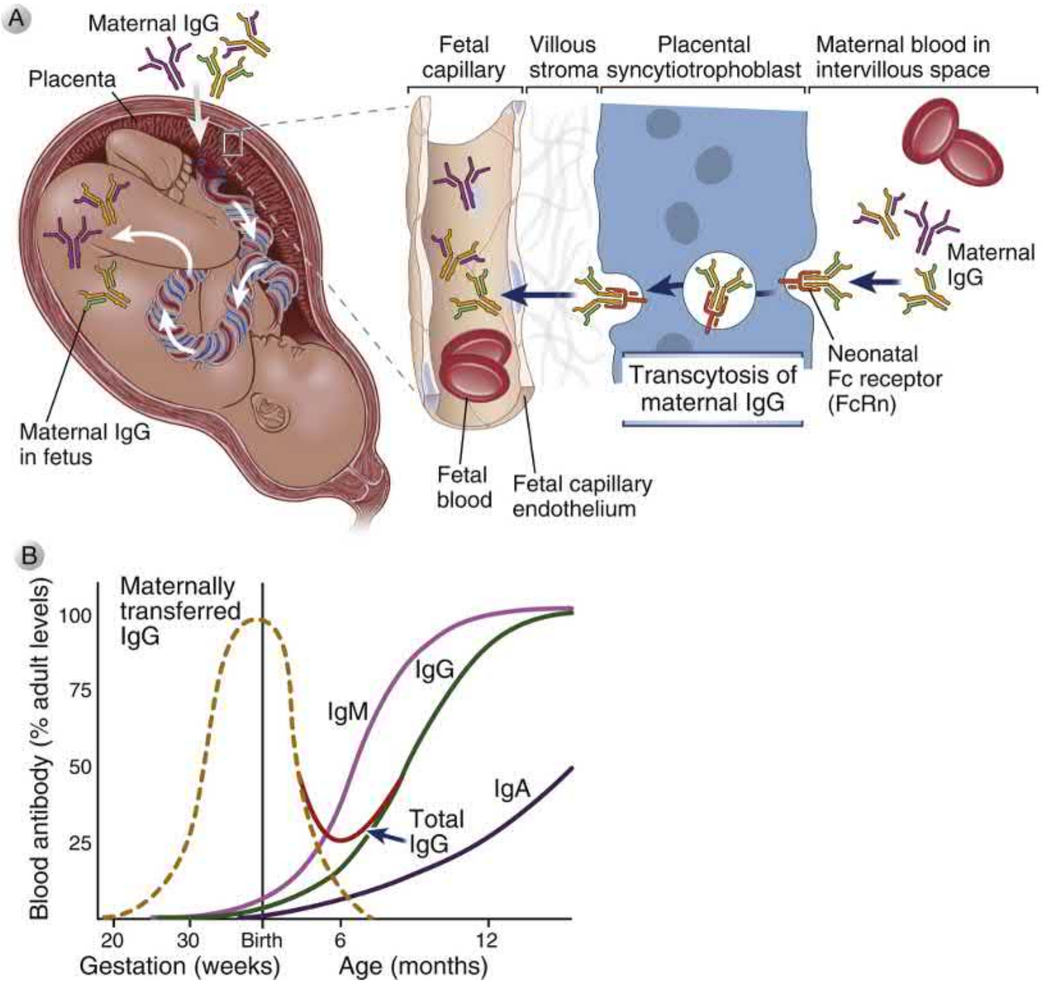

A
Maternal IgG
Placenta
Maternal IgG in fetus
Fetal capillary
Villous stroma
Placental syncytiotrophoblast
Maternal blood in intervillous space
Maternal IgG
Neonatal Fc receptor (FcRn)
Transcytosis of maternal IgG
Fetal blood
Fetal capillary endothelium
B
Blood antibody (% adult levels)
100
75
50
25
Maternally transferred IgG
IgG
IgM
IgA
Total IgG
20
30
Birth
6
12
Gestation (weeks)
Age (months)

FIGURE 14.11 Fetal and neonatal immunity mediated by passive transfer of maternal IgG. **A,** IgG is transported from maternal blood in the intervillous spaces of the placenta across the placental syncytial trophoblast cells by the neonatal Fc receptor *(FcRn),* and is then transported across fetal vessel endothelial cells into the fetal circulation. **B,** Serum immunoglobulin levels in the fetus and neonate. The neonate starts making IgG soon after birth, but the loss of maternal IgG and the slow increase in neonatally derived IgG results in a nadir of blood IgG concentration at about 6 months of age. The fetus starts making IgM late in pregnancy, and the IgM concentrations approach adult levels more quickly

in the baby than IgG. Nonetheless, these IgMs will not be specific for the wide range of microbes that the maternal IgGs will recognize, and the baby has a heightened risk for infection at the time of the IgG nadir. IgA levels in the baby take longer to reach adult levels than either IgM or IgG, but maternal IgA in breast milk can provide passive intestinal immunity in nursing babies.

Summary

- Regional immune systems, including those in the gastrointestinal tract, respiratory tract, and skin, are specialized collections of innate and adaptive immune cells at particular anatomic locations that perform protective and regulatory functions that are unique to those sites.
- The gastrointestinal immune system must cope with the presence of trillions of commensal bacteria in the gut lumen by preventing their invasion and tolerating their presence in the lumen, while also identifying and responding to numerically rare pathogenic organisms.
- Innate immunity in the gastrointestinal system is mediated by mucosal epithelial lining cells, which impede microbial invasion by tight intercellular junctions, secretion of mucus, and production of antimicrobial molecules such as defensins. Innate immune effector cells in the lamina propria include macrophages, DCs, ILCs, and mast cells. Intraepithelial lymphocytes, including γδ T cells, defend against commonly encountered microbes at the intestinal epithelial barrier.
- The adaptive immune system in the intestinal tract includes subepithelial collections of lymphoid tissues called gut-associated lymphoid tissues (GALT), such as the oropharyngeal tonsils, Peyer’s patches in the ileum, and

similar collections in the colon. M cells in the epithelial lining sample lumen antigens and transport them to antigen-presenting cells in the GALT. Lamina propria DCs extend processes through intestinal epithelial lining cells to sample luminal antigens.

- Effector B and T lymphocytes that differentiate from naive T cells in the GALT or mesenteric lymph nodes enter the circulation, and selectively migrate back to the intestinal lamina propria.
- Humoral immunity in the gastrointestinal tract is dominated by IgA secretion into the lumen, where the antibodies neutralize potentially invading pathogens. B cells in the GALT and mesenteric lymph nodes differentiate into IgA-secreting plasma cells through both T-dependent and T-independent mechanisms, and the plasma cells migrate to the lamina propria beneath the epithelial barrier and secrete IgA. Dimerized IgA is transported across the epithelium by the poly-Ig receptor and released into the lumen. IgA is also secreted into breast milk and mediates passive immunity in the gut of breast-feeding infants.
- Th17 cells in the intestinal tract secrete IL-17 interleukin-17 (IL-17) and IL-22, which enhance epithelial barrier function. Th2 cells are important in defense against intestinal parasites. Changes in bacterial flora influence the balance between different helper T cell subset responses, both in the gut and systemically.
- Immune responses to commensal organisms and food antigens in the lumen of the intestinal tract are minimized by selective expression of pattern recognition receptors on basolateral surfaces of the epithelial lining cells, and the generation of regulatory T cells that suppress adaptive immune responses. TGF-β, IL-10, and IL-2 are essential to maintain immune homeostasis in the bowel wall. Systemic tolerance to some antigens can be induced by feeding the antigens to mice, a phenomenon called oral tolerance.

- Mucosal immunity in the respiratory system defends against airborne pathogens and is the cause of allergic airway diseases such as asthma. Innate immunity in the bronchial tree depends on the mucus-producing, ciliated epithelial lining, which moves the mucus with entrapped microbes out of the lungs. Defensins, surfactant proteins, and alveolar macrophages provide antimicrobial and antiinflammatory functions. Treg and immunosuppressive cytokines are important for prevention of harmful responses to nonpathogenic organisms or other inhaled antigens.
- The cutaneous immune system defends against microbial invasion through the skin and suppresses responses against numerous commensal organisms. The epidermis provides a physical barrier to microbial invasion. Keratinocytes secrete defensins and inflammatory cytokines in response to microbial products. The dermis contains a mixed population of mast cells, macrophages, and DCs that respond to microbes and injury and mediate inflammatory responses.
- Skin DCs mediate innate immune responses and transport microbial and environmental antigens that enter through the skin to draining lymph nodes, where they initiate T cell responses. T cells activated in skin-draining lymph nodes express chemokine receptors and adhesion molecules that favor homing back to the skin.
- $CD4^+$ or $CD8^+$ effector memory cells generated in response to skin infections or commensals migrate to and stay in the dermis and epidermis for long periods. These resident memory cells have Th1, Th2, Th17, and CTL phenotypes, are important for defense against different types of skin-invading pathogens. Resident memory Tregs are also present in the skin and likely maintains tolerance to commensal skin organisms.

- Immune-privileged sites, which are tissues where immune responses are not readily initiated, include the brain, anterior chamber of the eye, and testis. The mechanisms of immune privilege include the tight junctions of endothelial cells in blood vessels, local production of immunosuppressive cytokines, and expression of cell surface molecules that inactivate or kill lymphocytes.
- Maternal immunologic tolerance to the developing mammalian fetus, which expresses allogeneic paternal antigens, depends on mechanisms that act locally at the placental maternal-fetal interface. Possible mechanisms include lack of MHC expression on fetal trophoblasts, the actions of Tregs, and the local IDO-mediated depletion of tryptophan needed for lymphocyte growth and generation of a toxic by-product.
- Neonatal protection against infections up to about 6 months of age is mediated by maternal IgG antibodies transferred to the fetal circulation through the placenta and in intestines of nursing babies by maternal IgA in ingested breast milk.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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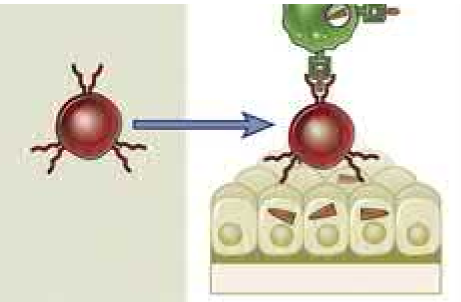
Chapter 15: Immunologic Tolerance and Autoimmunity



Overview Of Immunologic Tolerance,

T Lymphocyte Tolerance,
    Central T Cell Tolerance,
    Peripheral T Cell Tolerance,
    Factors That Determine the Tolerogenicity of Self Antigens,

B Lymphocyte Tolerance,
    Central B Cell Tolerance,
    Peripheral B Cell Tolerance,

Tolerance to Commensal Microbes and Other Foreign Antigens,
    Therapeutic Tolerance Induction,

Mechanisms Of Autoimmunity,
    General Features of Autoimmune Disorders,

  - Immunologic Abnormalities Leading to Autoimmunity,
  - Genetic Basis of Autoimmunity,
  - Role of Infections in Autoimmunity,
  - Other Factors in Autoimmunity,
- Summary,

Immunologic tolerance is defined as unresponsiveness to an antigen that is induced by exposure to that antigen. The term arose from the experimental observation that animals that had encountered an antigen under particular conditions would not respond to, that is, would tolerate, subsequent exposures to the same antigen. When specific lymphocytes encounter antigens, the lymphocytes may be activated, leading to immune responses, or the cells may be inactivated or eliminated, leading to tolerance. The same antigen may induce an immune response or tolerance, depending on the conditions of exposure and the presence or absence of other concomitant stimuli such as costimulators. Antigens that induce tolerance are called tolerogens, or tolerogenic antigens, to distinguish them from immunogens, which generate immunity. Tolerance to self antigens, also called **self-tolerance,** is a fundamental property of the normal immune system, and failure of self-tolerance results in immune reactions against self (autologous) antigens. Such reactions are called **autoimmunity,** and the diseases they cause are called **autoimmune diseases.** In Chapter 1, we introduced the concept of self–non-self discrimination, which is the ability of the immune system to recognize and respond to foreign antigens but not to self antigens. Macfarlane Burnet, among the first to hypothesize clonal selection, added the corollary that lymphocytes specific for self antigens are eliminated to prevent immune reactions against one’s own tissues. We now know there are multiple mechanisms of self-tolerance, in addition to elimination of self-reactive lymphocytes. Elucidating these mechanisms and why they sometimes fail is the key to understanding the pathogenesis of autoimmunity.

In this chapter, we will discuss immunologic tolerance mainly in the context of self-tolerance and how self-tolerance may fail, resulting in autoimmunity. We will also consider tolerance to foreign antigens and the potential of tolerance induction as a therapeutic strategy for allergic and autoimmune diseases. Induction of tolerance to prevent the rejection of cell and organ transplants is discussed in Chapter 17.

Overview of Immunologic Tolerance

There are several characteristics of tolerance in T and B lymphocyte populations. It is important to appreciate the general principles before we discuss the specific mechanisms of tolerance in these lymphocytes.

The mechanisms of tolerance eliminate and inactivate lymphocytes that express high-affinity receptors for self antigens. All individuals inherit essentially the same antigen receptor gene segments, and these recombine and are expressed in lymphocytes as the cells arise from precursor cells. The specificities of the receptors encoded by the recombined genes are random and are not influenced by what is foreign or self for each individual (see Chapter 8). It is not surprising that during this process of generating a large and diverse repertoire, some developing T and B cells in every individual may express receptors capable of recognizing normal molecules in that individual (i.e., self antigens). Therefore, there is a risk for lymphocytes to react against that individual's cells and tissues, causing disease. The mechanisms of immunologic tolerance prevent such reactions.

Tolerance is antigen specific, resulting from the recognition of antigens by individual clones of lymphocytes. This contrasts with therapeutic immunosuppression, which affects lymphocytes of many specificities. The key advances that allowed immunologists to study tolerance were the ability to induce this phenomenon in animals by exposure to defined antigens under various conditions and to then analyze the survival and functions of the lymphocytes that had encountered the antigens. In the 1950s, Peter Medawar and colleagues showed that neonatal mice of one strain exposed to cells from other strains became unresponsive to subsequent skin grafts from the donor strain. Later studies showed that tolerance could be induced not only to foreign cells but also to proteins and other antigens. Any antigen may be an immunogen or a tolerogen, depending on numerous factors, such as antigen exposure during lymphocyte maturation and recognition by specific lymphocytes in the presence or absence of innate immune responses. These factors are discussed later in the chapter.

Self-tolerance may be induced in immature self-reactive lymphocytes in the generative lymphoid organs (central tolerance) or in mature lymphocytes in peripheral sites (peripheral tolerance) (Fig. 15.1) **.** Central tolerance ensures that the repertoire of mature naive lymphocytes becomes incapable of responding to self antigens that are expressed in the generative lymphoid organs (the thymus for T cells and the bone marrow for B lymphocytes, also called primary or central lymphoid organs). However, central tolerance is not perfect, and many self-reactive lymphocytes complete their maturation and are present in healthy individuals. Therefore, the mechanisms of peripheral tolerance are needed to prevent activation of these potentially dangerous lymphocytes.

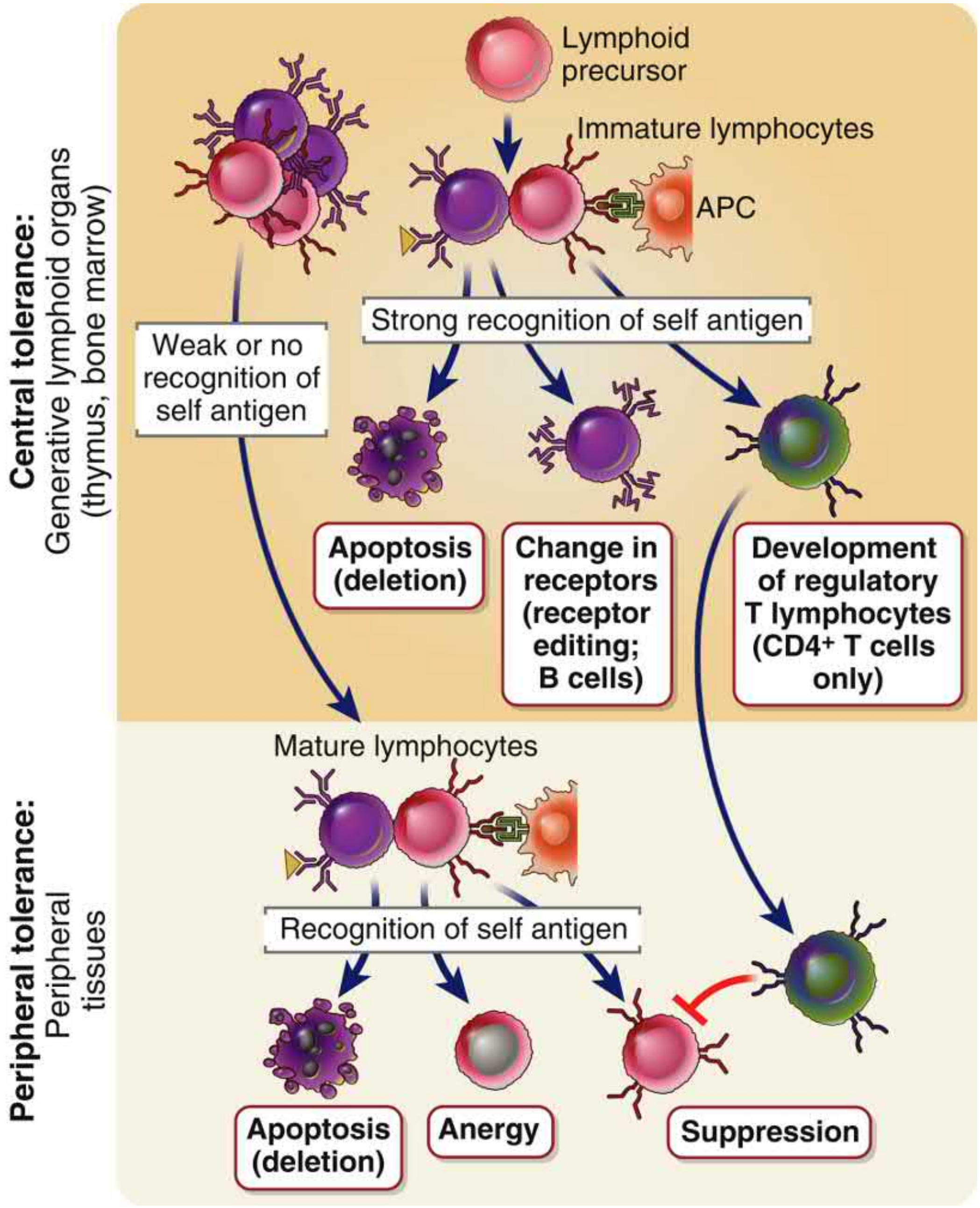

Central tolerance:
Generative lymphoid organs
(thymus, bone marrow)
Lymphoid precursor
Immature lymphocytes
APC
Strong recognition of self antigen
Weak or no recognition of self antigen
Apoptosis (deletion)
Change in receptors (receptor editing; B cells)
Development of regulatory T lymphocytes (CD4+ T cells only)
Peripheral tolerance:
Peripheral tissues
Mature lymphocytes
Recognition of self antigen
Apoptosis (deletion)
Anergy
Suppression

FIGURE 15.1 Central and peripheral tolerance to self antigens.In central tolerance, immature lymphocytes specific for self antigens may encounter these antigens in the generative lymphoid organs (referred to as central organs in the context of tolerance induction) and are deleted, change their specificity (B cells only), or (in the case of CD4$^+$ T cells) develop into

regulatory lymphocytes (Tregs). In peripheral tolerance, some self-reactive lymphocytes may mature and enter peripheral tissues and may be inactivated or deleted by an encounter with self antigens in these tissues or are suppressed by the regulatory T cells (Tregs, peripheral tolerance).

Central tolerance occurs during a stage in the maturation of lymphocytes when an encounter with an antigen may lead to cell death or replacement of a self-reactive antigen receptor with one that is not self-reactive. As lymphocytes are maturing in the generative lymphoid organs, immature cells may encounter antigens in these organs. The antigens that are present in these organs are mostly self antigens, because foreign (e.g., microbial) antigens that enter from the external environment are typically captured and taken to secondary lymphoid organs, such as the lymph nodes, spleen, and mucosal lymphoid tissues, and are not concentrated in the thymus or bone marrow. The antigens normally present in the thymus and bone marrow include ubiquitous, or widely disseminated, self antigens, some of which may be expressed by cells in the thymus and others may be brought in by the blood. In addition, many peripheral tissue–specific antigens are expressed in the thymus by a special mechanism that is described later. Therefore, in the generative lymphoid organs, the immature lymphocytes that recognize antigens are typically cells with high-affinity receptors specific for self antigens. The fates of immature lymphocytes that recognize self antigens with high affinity are described later (see Fig. 15.1).

Mature lymphocytes that recognize self antigens in peripheral tissues become incapable of activation by re-exposure to that antigen or die by apoptosis. These mechanisms of peripheral tolerance are important for maintaining unresponsiveness to self antigens that are expressed in peripheral tissues and not in the generative lymphoid organs and for tolerance to antigens that are expressed after mature lymphocytes specific for these antigens have already been generated.

Peripheral tolerance is also maintained by regulatory T cells (Tregs) that actively suppress the activation of lymphocytes specific for self and other antigens. Treg-mediated suppression occurs in secondary lymphoid organs and nonlymphoid tissues. In addition to suppressing immune responses to self antigens in peripheral tissues, Tregs may also prevent responses to antigens derived from commensal microbes, or from the fetus in pregnant females.

Some self antigens are sequestered from the immune system, and other antigens are ignored. Antigens may be sequestered from the immune system by anatomic barriers, such as in the testes and eyes, and thus cannot engage antigen receptors (see Chapter 14). Commensal microbes also may be separated from the immune system by physical barriers, such as the keratin layer of the skin and the mucus layer of the gastrointestinal and respiratory tracts. In experimental models, some self antigens are available for recognition by lymphocytes but, for unknown reasons, fail to elicit any response and are functionally ignored. The importance of this phenomenon of ignorance for the maintenance of self-tolerance is not established.

The induction of immunologic tolerance is a possible therapeutic strategy for preventing harmful immune responses in autoimmune and allergic disease and transplant rejection. Tolerance induction also may be useful for preventing immune reactions to the products of newly expressed genes in gene therapy protocols, for preventing reactions to injected proteins in patients with deficiencies of these proteins (e.g., patients with hemophilia treated with Factor VIII), and for promoting acceptance of stem cell transplants.

Experimental approaches, especially the creation of genetically modified mice, have provided valuable models for analysis of self-tolerance, and many of our current concepts are based on studies with such models. Furthermore, by identifying mutations and genetic polymorphisms that may be associated with autoimmunity in mice and humans, it has been possible to deduce some of the mechanisms of self-tolerance. However, for most self antigens, we do not know which ones induce central or peripheral tolerance (or are ignored). More importantly, it is also not known which tolerance mechanisms fail in common human autoimmune diseases, and this remains a major challenge in understanding autoimmunity.

We will discuss central and peripheral tolerance first in T cells and then in B lymphocytes, but many aspects of the processes are common to both lineages.

T Lymphocyte Tolerance

Much of our understanding of tolerance to self antigens is based on studying this process in T lymphocytes. This is, in part, because immunologists have developed informative experimental models for studying T cell tolerance. In addition, many of the therapeutic strategies that are being developed to induce tolerance to transplants and autoantigens are aimed at inactivating or eliminating T cells. This is because pathologic inflammatory reactions are typically mediated by T cells, and CD4$^+$ helper T cells also promote the production of potentially injurious antibodies by B cells.

Central T Cell Tolerance

During their maturation in the thymus, many immature T cells that recognize antigens with high avidity die, and some of the surviving cells in the CD4 $^+$ ***lineage develop into Tregs*** (Fig. 15.2) **.** Death of immature T cells as a result of recognition of antigens in the thymus is known as **deletion,** or **negative selection;** it was described in Chapter 8 in the discussion of T cell maturation. This process affects class I and class II major histocompatibility complex (MHC)-restricted T cells and is therefore important for tolerance in both CD8$^+$ and CD4$^+$ lymphocyte populations. Negative selection of thymocytes is responsible for the fact that the repertoire of mature

T cells that leave the thymus and populate peripheral lymphoid tissues is unresponsive to many self antigens that are present in the thymus. Negative selection occurs in double-positive T cells in the thymic cortex and newly generated single-positive T cells in the medulla. In both locations, immature thymocytes with high-affinity receptors for self antigens that encounter these antigens die by apoptosis. The two main factors that determine if a particular self antigen will induce negative selection of self-reactive thymocytes are the presence of that antigen in the thymus, by local expression or delivery by the blood, and the affinity of the thymocyte T cell receptors (TCRs) that recognize the antigen. Thus, the important questions that are relevant to negative selection are which self antigens are present in the thymus and how immature T cells that recognize these antigens are deleted.

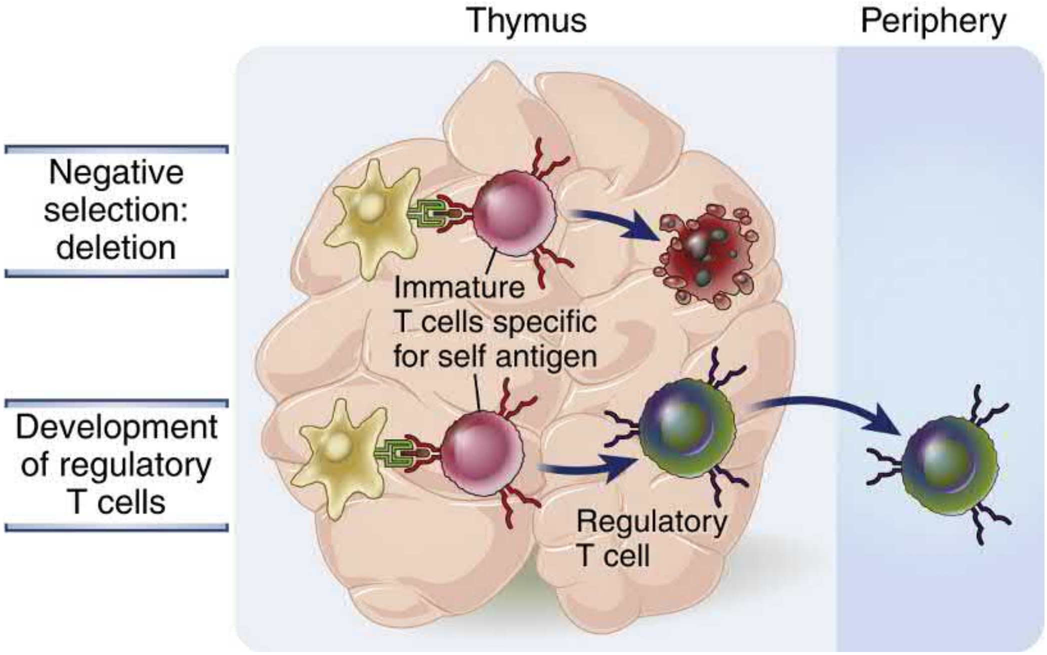

Thymus
Periphery
Negative selection: deletion
Immature T cells specific for self antigen
Development of regulatory T cells
Regulatory T cell

FIGURE 15.2 Central T cell tolerance.Recognition of self antigens by immature T cells in the thymus leads to the death of the cells (negative selection, or deletion) or to the development of regulatory T cells (Tregs) that enter peripheral tissues.

The antigens that are present in the thymus include many circulating and cell-associated proteins that are widely distributed in most tissues. The thymus also has a special mechanism for expressing numerous protein antigens that are not ubiquitously expressed but rather are limited to certain peripheral tissues, so that immature T cells specific for these antigens can be deleted from the developing T cell repertoire. These peripheral tissue antigens are produced in medullary thymic epithelial cells (MTECs) under the control of the **autoimmune regulator (AIRE)** protein. Mutations in the *AIRE* gene are the cause of an autoimmune disease called **autoimmune polyendocrine syndrome type 1 (APS1).** This group of diseases is characterized by antibody- and lymphocyte-mediated injury to multiple endocrine organs, including the parathyroids, adrenals, and pancreatic islets, as well as the skin and other tissues. A mouse model of APS1 has been developed by knockout of the *AIRE* gene, and it recapitulates many of the features of the human disease. Studies with mice have shown that several proteins that are produced in peripheral organs (such as pancreatic insulin) are also expressed at low levels in MTECs by an AIRE-dependent mechanism, and immature T cells that recognize these antigens are deleted in the thymus or the T cells develop into Tregs. In the absence of functional AIRE (as in APS1 patients and AIRE-knockout mice), these antigens are not expressed in the thymus, and $CD4^+$ and $CD8^+$ T cells specific for the antigens escape deletion, mature, and enter the periphery, where they attack the target tissues in which the antigens are expressed independent of AIRE (Fig. 15.3). Patients also make a variety of autoantibodies, probably because of failure to delete helper T cells specific for self antigens. Interestingly, some of these autoantibodies react against and neutralize their own interleukin-17 (IL-17), IL-22, and type I interferons (IFNs). The deficiency of IL-17 (and perhaps IL-22) makes these patients susceptible to mucocutaneous candidiasis, which is a common feature of APS1 and reflects the essential role of Th17 cytokines in defense against this fungal infection (see Chapter 10). The AIRE protein may function as a transcriptional regulator to promote the expression of selected tissue-restricted antigens in the thymus. It is a component of a multiprotein complex that is expressed mainly in MTECs and is involved in transcriptional elongation and chromatin remodeling. How AIRE drives expression of a wide range of tissue antigens and not other proteins is still not known. AIRE is also expressed in peripheral tissues, but its role in the elimination of self-reactive T cells in the periphery is not established,

High-affinity TCR signaling in immature T cells triggers the mitochondrial pathway of apoptosis. The mechanisms of apoptosis are described later in this chapter, when we discuss deletion as a mechanism of peripheral T cell tolerance. Clearly, immature and mature lymphocytes interpret antigen receptor signals differently—the former die and the latter are activated. The biochemical basis of this difference is not known.

Some self-reactive CD4 $^+$ T cells that see self antigens in the thymus are not deleted but instead differentiate into Tregs that are specific for these antigens (see Fig. 15.2) **.** The regulatory cells leave the thymus and inhibit responses against self antigens in the periphery. What determines the choice between deletion and development of Tregs is not known. Possible factors include the affinity of antigen recognition, the types of antigen-presenting cells (APCs) presenting the antigen, and the availability of certain cytokines locally in the thymus. We will describe the characteristics and functions of Tregs later in the context of peripheral tolerance because these cells suppress immune responses in the periphery.

Peripheral T Cell Tolerance

The mechanisms of peripheral tolerance are anergy (functional unresponsiveness), suppression by Tregs, and deletion (cell death) (Fig. 15.4) **.** These mechanisms may be responsible for T cell tolerance to tissue-specific self antigens, especially those that are not abundant in the thymus. We do not know if tolerance to different self antigens is maintained by one or another mechanism or if all of these mechanisms function cooperatively to prevent autoimmunity. The same mechanisms may also induce unresponsiveness to foreign antigens that are presented to the immune system under tolerogenic conditions.

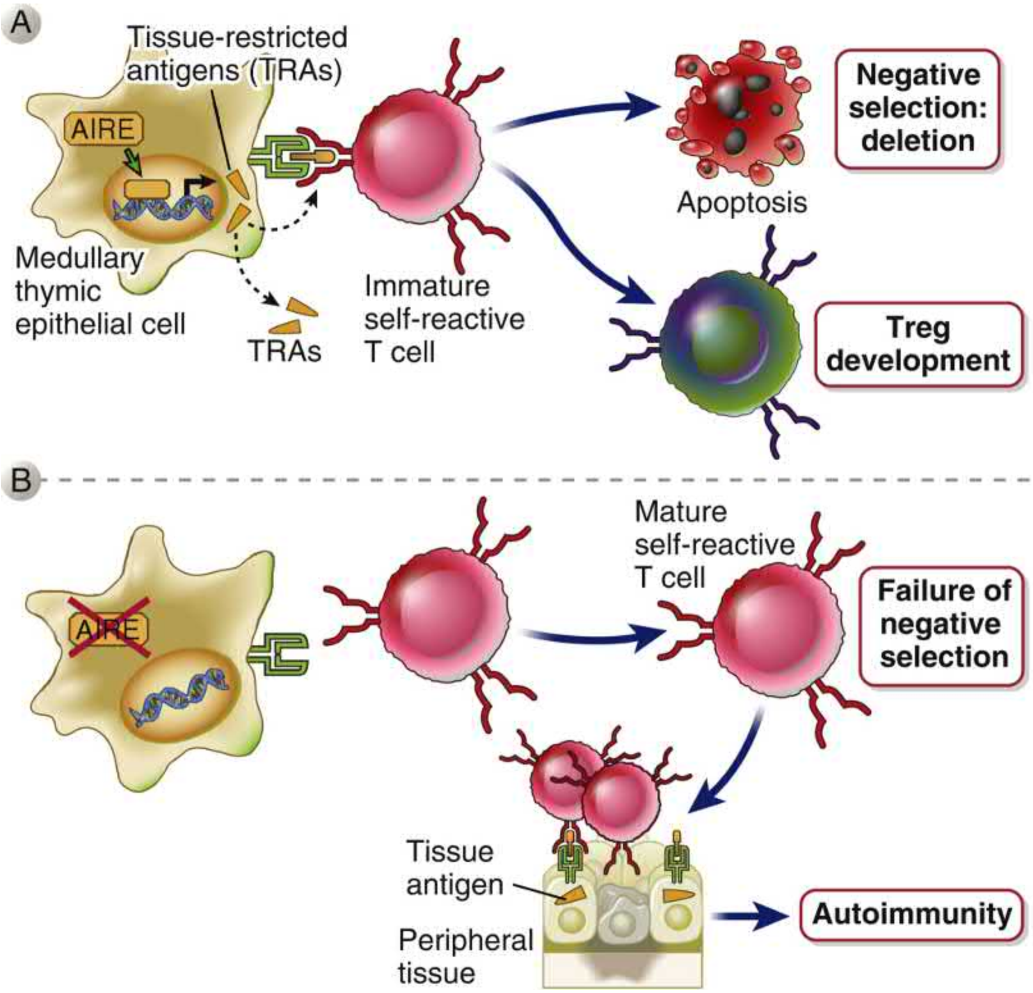

A
Tissue-restricted antigens (TRAs)
AIRE
Medullary thymic epithelial cell
TRAs
Immature self-reactive T cell
Apoptosis
Negative selection: deletion
Treg development
B
AIRE
Mature self-reactive T cell
Failure of negative selection
Tissue antigen
Peripheral tissue
Autoimmunity

FIGURE 15.3 The function of AIRE in deletion of T cells in the thymus. **A,** The autoimmune regulator *(AIRE)* protein is part of a complex that regulates the expression of tissue-restricted antigens *(TRAs)* in medullary thymic epithelial cells (MTECs). (Other proteins in this transcriptional regulatory complex are not shown.) Peptides derived from these antigens are displayed on the MTEC and recognized by immature antigen-specific T cells, leading to the deletion of many self-reactive T cells. **B,** In the absence of functional AIRE, these immature self-reactive T cells are not eliminated; they can mature into naive T cells that can be activated in secondary lymphoid organs, generating effector T cells *(not*

shown) that enter tissues where the antigens continue to be produced and cause injury. Although only CD4⁺ T cells are shown, AIRE also controls the deletion of CD8⁺ T cells.

Anergy (Functional Unresponsiveness)

Exposure of mature CD4 ⁺ ***T cells to an antigen in the absence of costimulation or innate immunity may make the cells incapable of responding to that antigen.*** In this process, which is called anergy, the self-reactive cells are not killed, but they become unresponsive to the antigen. We previously introduced the concept that full activation of T cells requires the recognition of the antigen by the TCR (which provides signal 1) and recognition of costimulators, mainly B7-1 and B7-2, by CD28 (signal 2) (see Chapter 9). Prolonged signal 1 (i.e., antigen recognition) alone may lead to anergy. It is likely that self antigens are continuously displayed to specific T cells in the absence of innate immunity and strong costimulation. Antigen-induced anergy has been demonstrated in a variety of experimental models, including studies with T cell clones exposed to antigens in vitro (which were the basis for the original definition of anergy), and in experiments in which antigens are administered to mice without adjuvants. Other studies have used transgenic mice in which particular protein antigens are expressed throughout life and are recognized by T cells in the absence of the inflammation and innate immune responses that normally accompany exposure to microbes. There is evidence that anergy is a mechanism of tolerance to some self antigens in humans as well. Anergic cells may survive for days or weeks in a quiescent state and then die.

Several mechanisms may function to induce and maintain the anergic state (Fig. 15.5):

- ***TCR-induced signal transduction is blocked in anergic cells.*** The mechanisms of this signaling block are not fully known. In different experimental models, it is attributable to decreased TCR expression (perhaps because of increased degradation; see later) and recruitment to the TCR complex of inhibitory molecules such as tyrosine phosphatases.

- ***Self antigen recognition without costimulation may activate cellular ubiquitin ligases, which ubiquitinate TCR-associated proteins and target them for proteolytic degradation in proteasomes or lysosomes.*** The net result is loss of these signaling molecules and defective T cell activation (see Fig. 7.22). One ubiquitin ligase that is important in T cells is CBL-b (see Chapter 7). Mice in which the gene encoding CBL-b is knocked out show spontaneous T cell proliferation and manifestations of autoimmunity, suggesting that this enzyme is involved in maintaining T cell unresponsiveness to self antigens. It is not known why self antigen recognition, which occurs typically without strong costimulation, activates these ubiquitin ligases, whereas foreign antigens that are recognized with costimulation do so much less or not at all.

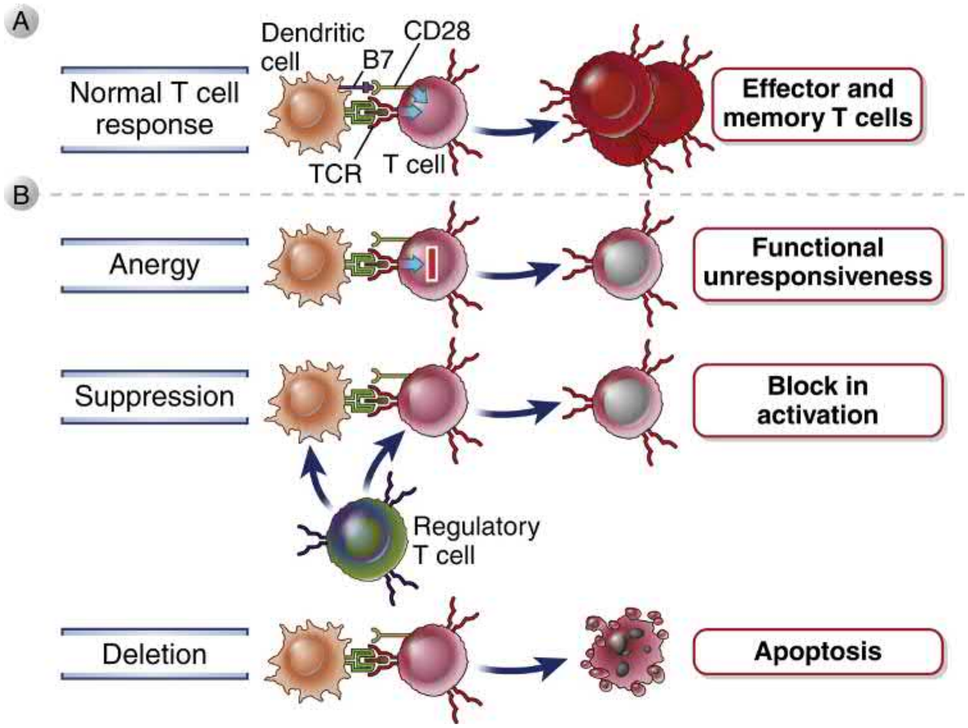

A
Normal T cell response
Dendritic cell
B7
CD28
TCR
T cell
Effector and memory T cells
B
Anergy
Functional unresponsiveness
Suppression
Block in activation
Regulatory T cell
Deletion
Apoptosis

FIGURE 15.4 Mechanisms of peripheral T cell tolerance. The signals involved in a normal immune response **(A)** and the three major mechanisms of peripheral T cell tolerance **(B)** are illustrated. *TCR,* T cell receptor.

- ***When T cells recognize self antigens in the absence of innate immune responses, they may engage inhibitory receptors of the CD28 family, whose function is to terminate T cell responses.*** The functions of the best-known inhibitory receptors of T cells are described in the following section.

Regulation of T Cell Responses by Inhibitory Receptors

In Chapter 9, we introduced the general concept that the outcome of antigen recognition by T cells is determined by a balance between engagement of activating and inhibitory receptors at the time of TCR recognition of antigen. Although many inhibitory receptors have been described, the two whose physiologic role in self-tolerance is best established are CTLA-4 and PD-1. These receptors have been called **coinhibitors,** to contrast them with costimulators. Studies of these inhibitory receptors have increased our understanding of tolerance mechanisms and led to new therapeutic approaches for manipulating immune responses.

CTLA-4

CTLA-4 (cytotoxic T lymphocyte antigen-4, so named because of how it was discovered) is a member of the CD28 receptor family (see Fig. 9.5) and, like the activating receptor CD28, it binds to B7 molecules. The importance of CTLA-4 in tolerance induction is illustrated by the finding that knockout mice lacking CTLA-4 and people with loss-of-function mutations in the *CTLA4* gene (discussed in more detail below) develop inflammatory lesions containing activated T cells and macrophages affecting multiple organs. These results indicate that defects in this one control mechanism result in failure of self-tolerance. Polymorphisms in the *CTLA4* gene are also associated with several autoimmune diseases in humans, including type 1 diabetes and Graves' disease.

CTLA-4 functions as a competitive inhibitor of CD28 and reduces the availability of B7 for the CD28 receptor (Fig. 15.6) **.** CTLA-4 has an unusual mechanism of action. It is expressed constitutively at high levels on Tregs and transiently on recently activated T cells, and it prevents the activation of responding T cells. In other words, CTLA-4 on one T cell (a Treg) can inhibit responses of other T cells. Recall that CD28 and CTLA-4 recognize the same ligands, B7-1 (CD80) and B7-2 (CD86) (see Fig. 9.5). CTLA-4 has a 10- to 20-fold higher affinity for B7 than does CD28. The cytoplasmic tail of CTLA-4 does not appear to have any signaling function; instead, it contains a motif that connects it to clathrin, a protein involved in receptor-mediated endocytosis. Because of this, CTLA-4 is an endocytic receptor that binds to B7 molecules on APCs and removes and ingests these molecules, a process that has been called transendocytosis (in which one cell endocytoses a protein from another cell). Therefore, when CTLA-4 is expressed on either Tregs or activated T cells, it out-competes CD28 and reduces the amount of B7 available on the APCs to provide costimulation via CD28. This competitive inhibition is especially important when B7 levels on APCs are low (as on resting APCs displaying self antigens). When B7 levels increase, for example, after exposure to microbes, there is relatively more engagement of the low-affinity receptor CD28. Because CTLA-4 limits the initial, costimulation-dependent activation of T cells in secondary lymphoid organs, mutating or blocking this receptor leads to severely dysregulated immune responses with enlarged lymph nodes, lymphoproliferation, and multiorgan inflammation.

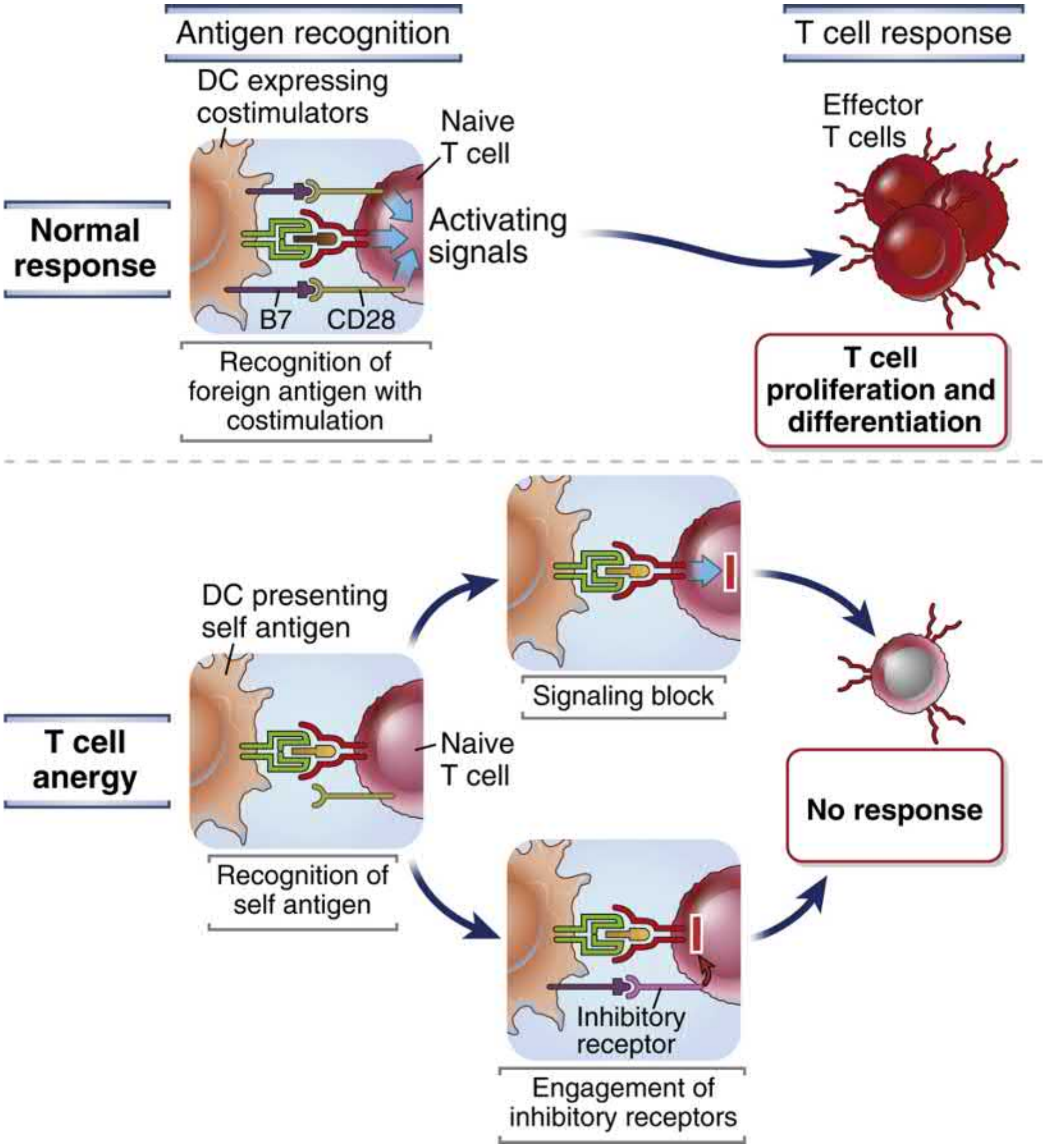

Antigen recognition
T cell response
DC expressing costimulators
Naive T cell
Normal response
Activating signals
Effector T cells
B7
CD28
Recognition of foreign antigen with costimulation
T cell proliferation and differentiation
DC presenting self antigen
Signaling block
T cell anergy
Naive T cell
No response
Recognition of self antigen
Inhibitory receptor
Engagement of inhibitory receptors

FIGURE 15.5 Mechanisms of T cell anergy. T cell responses are induced when the cells recognize an antigen presented by a professional antigen-presenting cell (APC) and activating receptors on the T cells (such as CD28) recognize costimulators on the APCs (such as B7). If the T cell recognizes a self antigen without costimulation, the T cell becomes unresponsive to the antigen because of a block in signaling from the T cell receptor (TCR) complex or engagement of inhibitory receptors (such as cytotoxic T

lymphocyte antigen 4 [CTLA-4] and programmed cell death protein-1 [PD-1]). The signaling block may be the result of recruitment of phosphatases to the TCR complex or the activation of ubiquitin ligases that degrade signaling proteins. The T cell remains viable but is unable to respond to the self antigen. *DC,* Dendritic cell.

Mutations of even one allele of CTLA-4 cause systemic inflammation involving multiple organs, with severe lymphoproliferation. It is thought that haploinsufficiency reduces the level of expressed CTLA-4 enough that it cannot compete effectively with CD28. Mutations affecting a protein called LRBA that is involved in the endocytosis and recycling of CTLA-4 cause a similar systemic inflammatory disease, further emphasizing the critical role of CTLA-4 trafficking in its inhibitory function. Because these diseases are caused by excessive B7-CD28–mediated costimulation resulting from failure of CTLA-4–mediated competition, they can be treated by blocking B7 molecules with the drug CTLA-4-Ig (see Fig. 9.7).

The realization that CTLA-4 limits, or acts as a checkpoint, in immune responses has led to the idea that lymphocyte activation can be promoted by reducing inhibition. Blocking CTLA-4 with antibodies results in increased immune responses to tumors, a process known as checkpoint blockade (see Chapter 18). Anti–CTLA-4 antibody is now approved for the treatment of advanced melanomas and other cancers. Predictably, many of the treated patients develop manifestations of autoimmunity with inflammation in various organs.

PD-1

Another inhibitory receptor of the CD28 family is PD-1 (programmed cell death protein 1, so called because it was originally thought to be involved in programmed cell death, but now is known not to have a role in T cell apoptosis). PD-1 recognizes two ligands, called PD-L1 and PD-L2; PD-L1 is expressed on APCs and many other tissue cells, and PD-L2 is expressed mainly on bone marrow–derived APCs. The receptor PD-1 is expressed on antigen-activated T cells. Engagement of PD-1 by either of its ligands leads to the phosphorylation of an immunoreceptor tyrosine-based inhibitory motif (ITIM) and a switch motif (ITSM) in the cytoplasmic tail (see Chapter 7). These motifs bind the phosphatase SHP2, which removes phosphates from various substrates (Fig. 15.7). Thus, PD-1 counteracts kinase-dependent signals from the TCR-coreceptor complex and from CD28 and other costimulatory receptors, resulting in inactivation of the T cells. Mice in which PD-1 is knocked out develop autoimmunity that is typically milder than that seen in CTLA-4 knockouts. PD-1 expression on T cells increases with antigen stimulation, so it is especially important for controlling responses to prolonged antigen exposure, as with self antigens, tumors, and chronic infections. Checkpoint blockade with anti–PD-1 and anti–PD-L1 antibodies is used to treat a wide variety of cancers, but it is also associated with the development of autoimmune reactions (see Chapter 18).

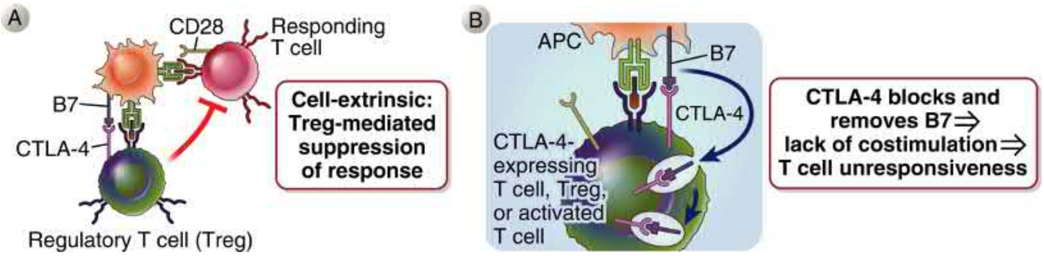

A
CD28
Responding T cell
B7
CTLA-4
Regulatory T cell (Treg)
Cell-extrinsic: Treg-mediated suppression of response
B
APC
B7
CTLA-4
CTLA-4-expressing T cell, Treg, or activated T cell
CTLA-4 blocks and removes B7⇒ lack of costimulation⇒ T cell unresponsiveness

FIGURE 15.6 Mechanism of action of CTLA-4. **A,** Cytotoxic T lymphocyte antigen 4 *(CTLA-4)* expressed on regulatory T cells *(Tregs)* or activated T cells can inhibit the activation of responding T cells on the same antigen-presenting cells *(APCs)* (in trans). **B,** CTLA-4 on regulatory or activated T cells binds to B7 molecules on APCs and removes these molecules from the surface of the APCs, making the B7 costimulators unavailable to CD28 and blocking T cell activation. This action of CTLA-4 is able to suppress immune responses best when B7 levels are low, enabling CTLA-4 to out-compete the lower-affinity receptor CD28.

Although both CTLA-4 and PD-1 establish checkpoints in immune responses, their roles may be complementary and not identical. For example, PD-1 appears to be most important for terminating the responses of effector T cells, especially CD8$^+$ cells, in peripheral tissues, whereas CTLA-4, as discussed previously, limits the initial activation of T cells in secondary lymphoid organs. Also, the prinicipal function of CTLA-4 is to prevent responses to self antigens, but PD-1 may have evolved to limit excessive reactions against microbes, especially viruses, and reduce immunopathology associated with these infections. Some of their major differences are summarized in Table 15.1.

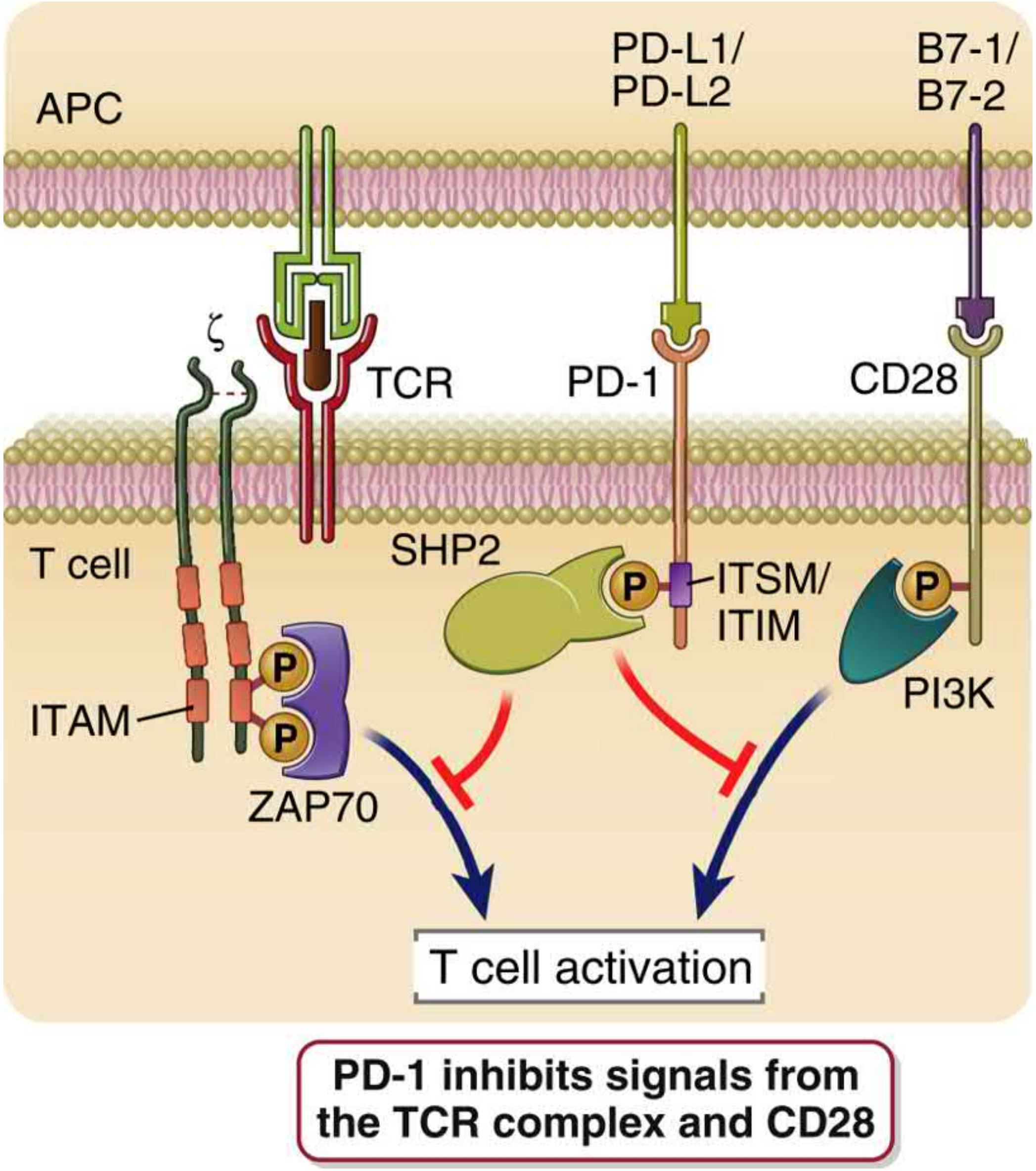

APC
PD-L1/
PD-L2
B7-1/
B7-2
ζ
TCR
PD-1
CD28
T cell
SHP2
P
ITSM/
ITIM
P
P
P
PI3K
ITAM
ZAP70
T cell activation
PD-1 inhibits signals from the TCR complex and CD28

FIGURE 15.7 Mechanism of action of PD-1.Engagement of its ligands, most often PD-L1, by PD-1 (programmed cell death protein-1) on T cells recruits a receptor-associated phosphatase, SHP2, to an ITIM or ITSM in the cytoplasmic tail of PD-1, which removes phosphates from other proteins and inhibits kinase-dependent signals from CD28 and the T cell receptor complex. Note that only some signals from the TCR complex and CD28 are shown. *APC,* Antigen-presenting cell;

ITAM, immunoreceptor tyrosine-based activation motif; *ITIM,* immunoreceptor tyrosine-based inhibitory motif; *ITSM,* immunoreceptor tyrosine-based switch motif; *PD-1,* programmed cell death protein-1; *TCR,* T cell receptor.

Table 15.1

Actions and Functions of CTLA-4 and PD-1

	CTLA-4	PD-1
Major site of action	Secondary lymphoid organs	Peripheral tissues
Stage of immune response that is inhibited	Induction (priming)	Effector phase
Cell type that is inhibited	$CD4^+ \geq CD8^+$	$CD8^+ > CD4^+$
Main cellular expression	Tregs, activated T cells	Activated T cells
Main signals inhibited	Competitive inhibitor of CD28 costimulation (by binding to B7 with high affinity and removing B7 from APCs)	Signaling inhibitor of CD28 and TCR: Inhibits kinase-dependent signals from CD28 and TCR by recruiting SHP2
Principal physiologic role (postulated)	Preventing autoimmune responses to self antigens	Preventing excessive (pathologic) responses to microbes (especially viruses) and some self antigens

APCs, Antigen-presenting cells; *CTLA-4,* cytotoxic T lymphocyte antigen 4; *PD-1,* programmed cell death protein 1; *TCR,* T cell receptor; *Tregs,* regulatory T cells.

Several other inhibitory receptors have been identified, including some belonging to the tumor necrosis factor (TNF) receptor family and others to the T cell immunoglobulin and mucin (TIM) family. The significance and biologic roles of these receptors are not as well established as the mechanisms of action and functions of CTLA-4 and PD-1. There is great interest in defining the roles of these receptors in the regulation of immune responses and in targeting these molecules therapeutically, especially for cancer immunotherapy (see Chapter 18).

Suppression by Regulatory T Cells

The concept that some lymphocytes could control the responses of other lymphocytes was proposed many years ago and was soon followed by experimental demonstrations of populations of T lymphocytes that suppressed immune responses. These initial findings led to enormous interest in suppressor T cells, which became one of the dominant topics of immunology research in the 1980s. However, this field has had a somewhat checkered history, mainly because attempts to define populations of suppressor cells and their mechanisms of action were largely unsuccessful. In the 1990s, the idea had an impressive rebirth, with the application of better approaches to define, purify, and analyze populations of T lymphocytes that inhibit immune responses. These cells are called regulatory T cells (Tregs).

Regulatory T cells are a subset of CD4$^+$ T cells whose principal function is to suppress immune responses (Fig. 15.8) **.** CD4$^+$ Tregs express high levels of the IL-2 receptor α chain (CD25) and the transcription factor called FOXP3. FOXP3 is a member of the forkhead family of transcription factors and is critical for the development and function of most Tregs. The essential role of FOXP3$^+$ Tregs in maintaining self-tolerance and preventing autoimmunity has been established by the analysis of inherited diseases. A rare systemic autoimmune disease in humans called **IPEX** (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome is caused by loss-of-function mutations in the

FOXP3 gene, which result in a deficiency of Tregs. This disease is fatal unless the children are given hematopoietic stem cell transplants, which restore normal Tregs. Mice with spontaneous or experimentally induced mutations in the *foxp3* gene develop a similar multisystem autoimmune disease associated with an absence of $CD25^+$ Tregs, and the disease can be prevented by introducing normal Tregs. These observations have established the importance of Tregs for maintaining self-tolerance. The recent surge of interest in Tregs is because of an increasing appreciation of their physiologic roles, as well as the possibility that defects in these cells may result in various autoimmune diseases and, conversely, that Tregs can be administered or expanded to treat inflammatory diseases.

Phenotypic Markers and Heterogeneity of Regulatory T Cells

Although numerous T cell populations have been described as possessing suppressive activity, the cell type whose regulatory role is best established is $CD4^+$ $FOXP3^+$ $CD25^{high}$. FOXP3 and CD25 are essential for the generation, maintenance, and function of these cells. These cells usually express low levels of the receptor for IL-7 (CD127), and as predicted from this pattern of receptor expression, they use IL-2 but not IL-7 as their growth and survival factor. $FOXP3^+$ Tregs typically express high levels of CTLA-4, which is also required for their function. Demethylation of the *FOXP3* gene locus and of other loci containing genes that are expressed in these cells serves to maintain a stable regulatory T cell phenotype, and these epigenetic changes are now used to identify Tregs in basic and clinical research. $FOXP3^+$ Tregs are classified as thymic or peripheral, based on where they develop (discussed next). There also may be subsets of Tregs that are distinguished by history of antigen response (naive, activated, and memory) or tissue residence (lymphoid and nonlymphoid). Tregs that are located in peripheral tissues, such as the intestines, lungs, skin, and fat, may have functions that are unique to each tissue (discussed later). Tregs have been described in the follicles of secondary lymphoid organs, where they may inhibit antibody responses. In different types of immune responses, Tregs may be induced to express the same chemokine receptors as effector T cells. This is one way in which Tregs colocalize in tissues with effector cells, enabling the Tregs to control diverse immune reactions in tissues.

Generation and Maintenance of Regulatory T Cells

Tregs are generated mainly by self antigen recognition in the thymus and also by recognition of self and foreign antigens in peripheral tissues. In the thymus, development of Tregs is one of the fates of T cells committed to the CD4 lineage that recognize self antigens. In peripheral tissues, antigen recognition in the absence of strong innate immune responses favors the generation of regulatory cells from naive $CD4^+$ T lymphocytes; Tregs can also develop after inflammatory reactions. The majority of Tregs in lymphoid tissues are thought to be derived from the thymus as a consequence of self antigen expression. Although many markers have been proposed to distinguish thymic from peripheral Tregs, whether these markers can be used to reliably distinguish the two subsets in lymphoid organs or blood of mice and humans has not been established.

The generation, survival, and functional competence of Tregs are dependent on the cytokine IL-2. Patients with homozygous mutations in the IL-2 receptor α or β chain develop systemic autoimmunity and have few functional Tregs. Mice in which the gene for IL-2 or for the α or β chain of the IL-2 receptor is knocked out also develop autoimmunity, manifested by inflammatory bowel disease (IBD), autoimmune hemolytic anemia, and multiple autoantibodies (including anti-erythrocyte and anti-DNA antibodies). These mice have greatly reduced numbers of $CD25^+$ $FOXP3^+$ Tregs, and their disease can be corrected by injecting these cells. IL-2 promotes differentiation of T cells in the thymus into the regulatory subset and is required for the maintenance of this cell population in the periphery. Because $FOXP3^+$ Tregs do not produce IL-2, this growth factor is provided by conventional T cells responding to self or foreign antigens (Fig. 15.9). IL-2 activates the transcription factor STAT5, which may enhance expression of FOXP3 as well as other genes that are involved in the function of Tregs. These results are the basis for ongoing clinical trials testing the ability of IL-2 to promote Tregs in humans for the control of graft-versus-host disease, autoimmune inflammation, and graft rejection.

The generation of some Tregs requires the cytokine transforming growth factor-β (TGF-β). Culture of naive T cells with activating anti-TCR antibodies together with TGF-β (and IL-2, discussed earlier) can induce the development of regulatory cells in vitro. In mice, elimination of TGF-β or blocking of TGF-β signals in T cells leads to a systemic inflammatory disease because of uncontrolled leukocyte activation. TGF-β stimulates expression of FOXP3, and this could be its major role in Treg development.

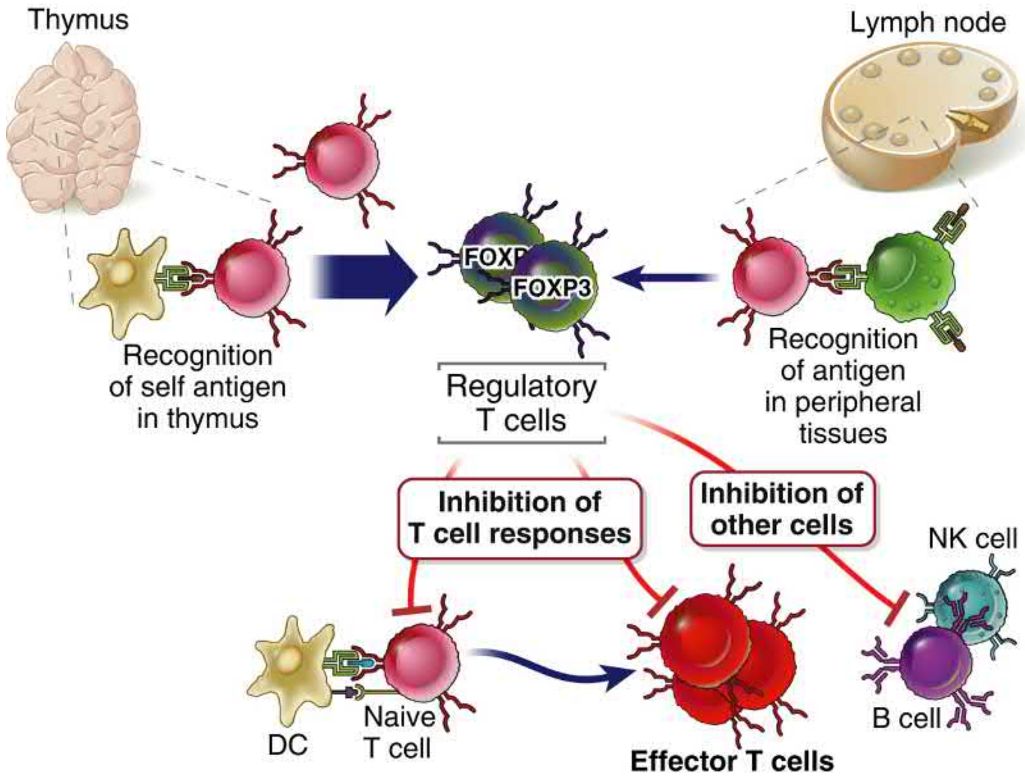

Thymus
Lymph node
FOXP3
FOXP3
Recognition of self antigen in thymus
Regulatory T cells
Recognition of antigen in peripheral tissues
Inhibition of T cell responses
Inhibition of other cells
NK cell
B cell
DC
Naive T cell
Effector T cells

FIGURE 15.8 Regulatory T cells.Regulatory T cells (Tregs) are generated by self antigen recognition in the thymus (sometimes called natural regulatory cells) and (probably to a lesser extent) by antigen recognition in peripheral lymphoid organs (called inducible or adaptive regulatory cells). The development and survival of these Tregs require interleukin-2 and the transcription factor FOXP3. In peripheral tissues, Tregs suppress the activation and effector functions of other self-reactive and potentially pathogenic lymphocytes. *DC,* Dendritic cell; *NK,* natural killer.

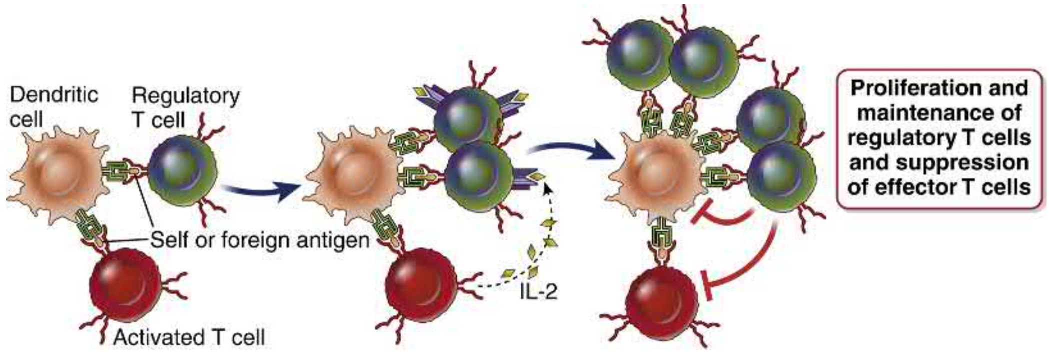

Dendritic cell
Regulatory T cell
Self or foreign antigen
Activated T cell
IL-2
Proliferation and maintenance of regulatory T cells and suppression of effector T cells

FIGURE 15.9 Role of interleukin-2 in the maintenance of regulatory T cells.Interleukin-2 *(IL-2)* produced by conventional T cells responding to self or foreign antigens acts on regulatory T cells (Tregs) recognizing the antigen on antigen-presenting dendritic cells and promotes the survival and function of the Tregs, enabling them to control the responses of the conventional T cells.

Mechanisms of Action of Regulatory T Cells

Tregs appear to suppress immune responses at multiple steps—at the induction of naive T cell activation in lymphoid organs as well as the effector phase of these responses in tissues. They control T cell responses mainly by inhibiting the stimulatory ability of dendritic cells (DCs), and also by suppressing T cell activation. Tregs may also suppress B cell activation and inhibit the proliferation and differentiation of natural killer (NK) cells. Although numerous mechanisms of suppression have been proposed, the following are the best supported by available data.

- ***CTLA-4–mediated inhibition of costimulation.*** CTLA-4 on Tregs binds to B7 molecules on APCs and removes these molecules, resulting in reduced availability of B7 for CD28-mediated costimulation (see Fig. 15.6).
- ***Production of the immunosuppressive cytokines IL-10 and TGF-β.*** These cytokines are discussed below.

- ***Consumption of IL-2.*** Because of the high level of expression of the IL-2 receptor, these cells may absorb IL-2 and deprive other cell populations of this growth factor, resulting in reduced proliferation and differentiation of other IL-2–dependent cells.

Whether all regulatory cells work by all of these mechanisms or if there are subpopulations that use different mechanisms to control immune responses has not been established. In fact, there is some evidence in humans that two different populations of Tregs can be distinguished by the expression of FOXP3 or production of IL-10, but this separation may not be absolute.

Inhibitory Cytokines Produced by Regulatory T Cells

TGF-β and IL-10 are involved in the generation and functions of Tregs. These cytokines are produced by and act on many other cell types in addition to regulatory cells. Here we describe the properties and actions of these cytokines.

Transforming Growth Factor-β

TGF-β was discovered as a protein made by tumors that promoted the survival of cancer cells in vitro. It is actually a family of closely related molecules encoded by distinct genes, commonly designated TGF-β1, TGF-β2, and TGF-β3. Cells of the immune system synthesize mainly TGF-β1. TGF-β1 is produced by $CD4^+$ Tregs, activated macrophages, and many other cell types. The processing of TGF-β, TGF-β receptors, and signaling downstream of these receptors were described in Chapter 7.

TGF-β has many important and diverse roles in the immune system:

- ***TGF-β inhibits the proliferation and effector functions of T cells and the activation of macrophages.*** TGF-β inhibits classical macrophage activation but is one of the cytokines secreted by alternatively activated macrophages (see

Chapter 10). TGF-β also suppresses the activation of other cells, such as neutrophils and endothelial cells. By these inhibitory actions, TGF-β functions to control immune and inflammatory responses.

- ***TGF-β regulates the differentiation of functionally distinct subsets of T cells.*** As described earlier, TGF-β stimulates the development of peripheral FOXP3$^+$ Tregs, best established in vitro. In combination with cytokines produced during innate immune responses, such as IL-1 and IL-6, TGF-β promotes the development of the Th17 subset of CD4$^+$ T cells by virtue of its ability to induce the transcription factor RORγt (see Chapter 10). The ability of TGF-β to suppress immune and inflammatory responses, in part by generating Tregs, and to promote the development of proinflammatory Th17 cells in the presence of other cytokines, is an interesting example of how a single cytokine can have diverse and sometimes opposing actions depending on the context in which it is produced. TGF-β can also inhibit development of Th1 and Th2 subsets.
- ***TGF-β stimulates production of immunoglobulin A (IgA) antibodies by inducing B cells to switch to this isotype.*** IgA is the major antibody isotype required for mucosal immunity (see Chapter 14).
- ***TGF-β promotes tissue repair after local immune and inflammatory reactions subside.*** This function is mediated mainly by the ability of TGF-β to stimulate collagen synthesis and matrix-modifying enzyme production by macrophages and fibroblasts and by promoting angiogenesis. This cytokine may play a pathologic role in diseases in which fibrosis is an important component, such as pulmonary fibrosis and systemic sclerosis.

Interleukin-10

IL-10 is an inhibitor of activated macrophages and DCs and is thus involved in the control of innate immune reactions and cell-mediated immunity. It is a member of a family of heterodimeric cytokines that include IL-22, and others. The IL-10 receptor belongs to the type II cytokine receptor family (similar to the receptor for IFNs) and consists of two chains, which associate with the Janus family kinases JAK1 and TYK2 and activate STAT3. IL-10 is produced by many immune cell populations, including activated macrophages and DCs, Tregs, and Th1 and Th2 cells. Because it is both produced by and inhibits macrophages and DCs, it functions as a negative feedback regulator. IL-10 is also produced by some B lymphocytes, which have been shown to have immune suppressive functions and have been called regulatory B cells.

The biologic effects of IL-10 result from its ability to inhibit many of the functions of activated macrophages and DCs.

- ***IL-10 inhibits the production of IL-12 by activated DCs and macrophages.*** Because IL-12 is a critical stimulus for IFN-γ secretion, which plays an important role in innate and adaptive cell-mediated immune reactions against intracellular microbes, IL-10 suppresses all such reactions. In fact, IL-10 was first identified as a cytokine that inhibited IFN-γ production.
- ***IL-10 inhibits the expression of costimulators and class II MHC molecules on DCs and macrophages.*** Because of these actions, IL-10 inhibits T cell activation and terminates cell-mediated immune reactions.

Infants who have homozygous loss-of-function mutations in the *IL10* gene or in the gene for the IL-10 receptor develop severe colitis before the age of 1 year. Knockout mice lacking IL-10 in all cells or only in Tregs also develop colitis, probably as a result of uncontrolled activation of lymphocytes and macrophages reacting to enteric microbes. Because of these findings, it is thought that this cytokine is especially important for controlling inflammatory reactions in mucosal tissues, particularly the gastrointestinal tract (see Chapter 14).

The Epstein-Barr virus contains a gene homologous to human IL-10, and viral IL-10 has the same activities as the natural cytokine. This raises the intriguing possibility that the virus has acquired the IL-10–like gene during its evolution, giving it the ability to inhibit host immunity and thus a survival advantage in the infected host.

Roles of Regulatory T Cells in Self-Tolerance and Autoimmunity

The elucidation of the genetic basis of IPEX syndrome and the similar disease in mice caused by mutations in the *foxp3* gene, described earlier, is convincing proof of the importance of Tregs in maintaining self-tolerance and homeostasis in the immune system. Numerous attempts are being made to identify defects in the development or function of Tregs in more common autoimmune and inflammatory diseases in humans such as IBD, type 1 diabetes, and multiple sclerosis (MS), as well as in allergic disorders. Defects in Tregs or resistance of effector cells to suppression by Tregs may contribute to the pathogenesis of these diseases. However, it has proved difficult to definitively establish the role of Treg defects in these common diseases, in part because defining and quantifying functional Tregs in humans is challenging.

There is also potential for expanding Tregs in culture and injecting them back into patients to control pathologic immune responses. Clinical trials of Treg transfer are ongoing in attempts to treat transplant rejection, graft-versus-host disease, and autoimmune and other inflammatory disorders. In addition, clinical trials are underway to expand these cells in patients by administering the cytokine IL-2 in doses or forms that preferentially bind to CD25 and thus activate Tregs.

In addition to their importance for controlling autoimmunity, Tregs have been shown to serve other roles. Subpopulations of Tregs with unique transcriptional signatures are present in many tissues and appear to perform functions that are especially beneficial for those tissues. Tregs that reside in skin, muscle, and organs such as the lung promote tissue repair and the proliferation and differentiation of stem cells, thus helping to restore tissue integrity after inflammatory reactions resolve. Adipose tissue Tregs control fat metabolism. Tregs are also involved in maintaining fetal tolerance and preventing the rejection of fetuses and play a role in preventing inflammatory responses to commensal microbes (see Chapter 14).

Deletion of T Cells by Apoptotic Cell Death

T lymphocytes that recognize self antigens with high affinity or are repeatedly stimulated by antigens may die by apoptosis. There are two major pathways of apoptosis (Fig. 15.10), both of which have been implicated in peripheral deletion of mature T cells.

- The **mitochondrial** (or **intrinsic**) **pathway** is regulated by the BCL-2 family of proteins, named after the founding member, BCL-2, which was discovered as an oncogene in a B cell lymphoma and shown to inhibit apoptosis. Some members of this family are pro-apoptotic and others are anti-apoptotic. The pathway is initiated when cytoplasmic proteins of the BCL-2 family that belong to the BH3-only subfamily (so called because they contain one domain that is homologous to the third conserved domain of BCL-2) are induced or activated as a result of growth factor deprivation, noxious stimuli, DNA damage, or certain types of receptor-mediated signaling (e.g., strong signals delivered by self antigens in immature lymphocytes). BH3-only proteins are sensors of cell stress that bind to and influence death effectors and regulators. In lymphocytes, the most important of these sensors is a protein called BIM. Activated BIM binds to two pro-apoptotic effector proteins of the BCL-2 family called BAX and BAK, which oligomerize and insert into the outer mitochondrial membrane, leading to increased mitochondrial permeability. Growth factors and other survival signals induce the expression of anti-apoptotic members of the BCL-2 family, such as BCL-2 and BCL-X_L, which function as inhibitors of apoptosis by blocking BAX and BAK and thus maintaining intact mitochondria. BH3-only proteins also antagonize BCL-2 and BCL-X_L. When cells are deprived of survival signals, the mitochondria become leaky because of the actions of the BH3-only protein sensors and BAX and BAK effectors and the relative deficiency of anti-apoptotic proteins such as BCL-2 and BCL-X_L. The result is that many mitochondrial components, including cytochrome *c,* leak out into the cytosol and activate cytosolic enzymes called **caspases.** Cytochrome *c* binds to a cytosolic protein called APAF-1, which then oligomerizes and activates procaspase-9, yielding active caspase-9. Caspase-9 in turn cleaves and thereby activates downstream caspases that induce nuclear DNA fragmentation and other changes that culminate in apoptotic death.

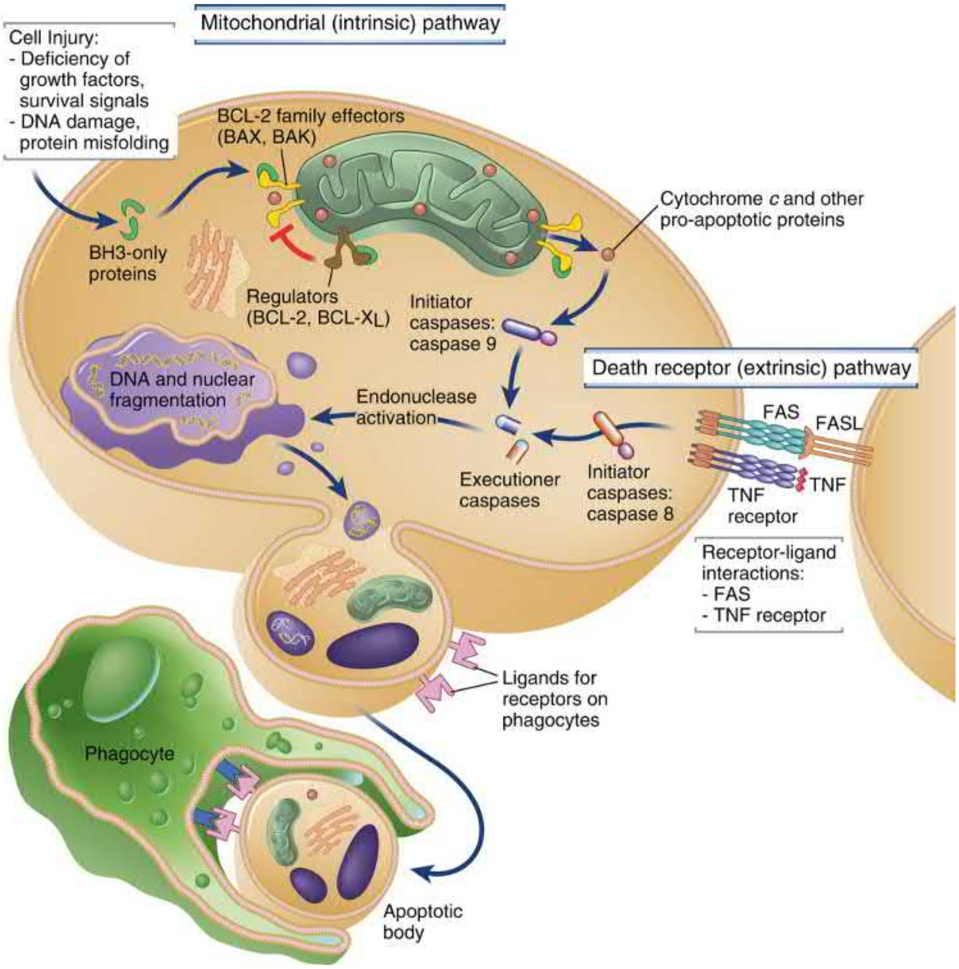

Mitochondrial (intrinsic) pathway
Cell Injury:
- Deficiency of growth factors, survival signals
- DNA damage, protein misfolding
BCL-2 family effectors (BAX, BAK)
Cytochrome *c* and other pro-apoptotic proteins
BH3-only proteins
Regulators (BCL-2, BCL-XL)
Initiator caspases: caspase 9
Death receptor (extrinsic) pathway
DNA and nuclear fragmentation
Endonuclease activation
FAS
FASL
TNF
Executioner caspases
Initiator caspases: caspase 8
TNF receptor
Receptor-ligand interactions:
- FAS
- TNF receptor
Ligands for receptors on phagocytes
Phagocyte
Apoptotic body

FIGURE 15.10 Pathways of apoptosis.Apoptosis is induced by the mitochondrial and death receptor pathways, described in the text, which culminate in fragmentation of the dead cell and phagocytosis of apoptotic bodies. *TNF,* Tumor necrosis factor.

- In the **death receptor** (or **extrinsic**) **pathway,** cell surface receptors of the TNF receptor superfamily are engaged by their TNF superfamily ligands. The receptors oligomerize and activate cytoplasmic adaptor proteins, which assemble procaspase-8, which cleaves itself when oligomerized and produces active caspase-8. The active caspase-8 cleaves downstream caspases, again resulting in apoptosis. In T cells the most important death receptor is FAS (CD95), and its ligand is FAS ligand (FASL). FAS is a member of the TNF receptor family, and FASL is homologous to TNF. In many cell types, caspase-8 cleaves and activates a BH3-only protein called BID that binds to BAX and BAK and induces apoptosis via the mitochondrial pathway. Thus, the mitochondrial pathway may serve to amplify death receptor signaling.

Table 15.2

Factors That Determine the Immunogenicity and Tolerogenicity of Protein Antigens

	Features That Favor Stimulation of Immune Responses	Features That Favor Tolerance
Persistence	Short-lived (eliminated by immune response)	Prolonged, leading to persistent antigen receptor engagement
Portal of entry; location	Subcutaneous, intradermal; absence from generative organs	Intravenous, mucosal; presence in generative organs
Presence of adjuvants	Antigens with adjuvants: Induce costimulators and cytokines	Antigens without adjuvants: Low levels of costimulators and cytokines
Properties of APCs	Mature dendritic cells: High levels of costimulators	Immature (resting) dendritic cells: Low levels of costimulators and cytokines

APCs, Antigen-presenting cells.

In cells undergoing apoptosis, fragments of the nucleus and cytoplasm break off in membrane-bound structures called apoptotic bodies. There are also biochemical changes in the plasma membrane, including the exposure of lipids such as phosphatidylserine, which is normally on the inner face of the plasma membrane. These alterations are recognized by receptors on phagocytes, and apoptotic bodies and cells are rapidly engulfed and eliminated, without ever having elicited a host inflammatory response. This process is called efferocytosis. Furthermore, phagocytosis of apoptotic cells may induce the production of antiinflammatory mediators by macrophages.

The best evidence for the involvement of the two apoptotic pathways in the elimination of mature self-reactive lymphocytes is that genetic ablation of either in mice results in systemic autoimmunity, and the disease is more severe if both pathways are disabled. Mutations of FAS, FASL, or downstream caspases cause a human autoimmune disease called **autoimmune lymphoproliferative syndrome (ALPS).** These two death pathways may function in different ways to maintain self-tolerance.

- T cells that recognize self antigens in the absence of costimulation may induce apoptosis by the mitochondrial pathway. In normal immune responses, the responding lymphocytes receive signals from the TCR, costimulators, and growth factors. These signals stimulate the expression of anti-apoptotic proteins of the BCL-2 family (BCL-2, BCL-X_L) and thus prevent apoptosis and promote cell survival, the necessary prelude to proliferation. When T cells avidly recognize self antigens, they may activate BIM, which triggers death by the mitochondrial pathway, as described earlier. At the same time, because of the relative lack of costimulation and growth factors, the anti-apoptotic members of the BCL-2 family remain at low levels, and the actions of the effectors BAX and BAK are thus not counteracted. The BIM-dependent mitochondrial pathway of apoptosis is also involved in negative selection of self-reactive T cells in the thymus (described earlier) and in the contraction phase (decline) of immune responses after the initiating antigen has been eliminated (see Chapter 9).

- Repeated stimulation of T cells results in coexpression of the death receptor FAS and its ligand FASL, and engagement of FAS triggers apoptotic death. When T cells are repeatedly activated, both FAS and FASL are expressed on the cell surface, and binding of the ligand to the receptor activates a cascade of caspases, which ultimately cause the apoptotic death of the cells. The same pathway of apoptosis may be involved in the elimination of self-reactive B lymphocytes also in the periphery (discussed later).

Factors That Determine the Tolerogenicity of Self Antigens

Studies with a variety of experimental models have shown that many features of protein antigens determine whether these antigens will induce T cell activation or tolerance (Table 15.2). Self antigens have several properties that make them tolerogenic. These antigens are expressed in generative lymphoid organs, where they are recognized by immature lymphocytes. In peripheral tissues, self antigens engage antigen receptors of specific lymphocytes for prolonged periods and without inflammation or innate immunity.

The nature of the DC that displays antigens to T lymphocytes is an important determinant of the subsequent response. DCs that are resident in lymphoid organs and nonlymphoid tissues may present self antigens to T lymphocytes and maintain tolerance. Tissue DCs are normally in a resting (immature) state and express low levels of costimulators; some of them may traffic at a low rate from epithelia to secondary lymphoid organs even at steady state (in the absence of infection or inflammation). Such APCs may be constantly presenting self antigens without providing strong costimulation, and T cells that recognize these antigens become anergic or differentiate into Tregs instead of effector and memory lymphocytes. By contrast, DCs that are activated by microbes are the principal APCs for initiating T cell responses (see Chapter 6). As we will discuss later, local infections and inflammation may activate resident DCs, leading to increased expression of costimulators, breakdown of tolerance, and autoimmune reactions against tissue antigens. There is great interest in manipulating the properties of DCs as a way of enhancing or inhibiting immune responses for therapeutic purposes.

Our understanding of the mechanisms that link the signals that a T cell receives at the time of antigen recognition with the fate of that T cell remains incomplete. These concepts are based largely on experimental models in which antigens are administered to mice or are produced by transgenes expressed in mice. One of the continuing challenges in this field is to define the mechanisms by which various natural (normally expressed) self antigens induce tolerance, especially in humans.

B Lymphocyte Tolerance

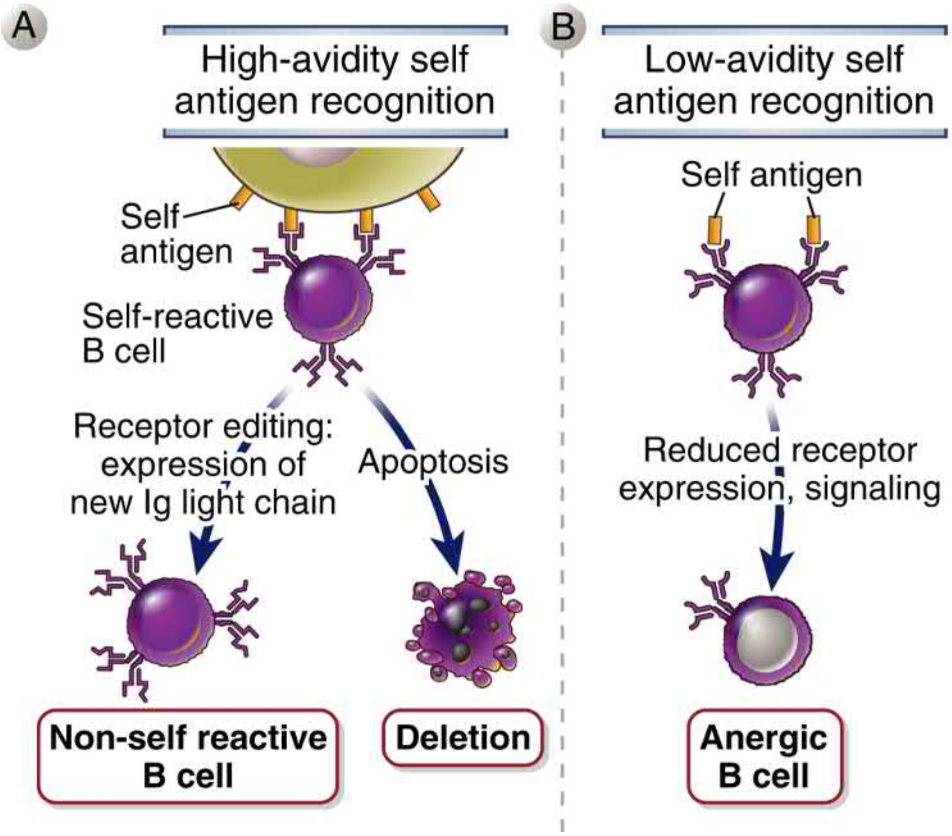
Tolerance in B lymphocytes is necessary for maintaining unresponsiveness to T-independent self antigens, such as polysaccharides and lipids. B cell tolerance also plays a role in preventing antibody responses to protein antigens. Experimental studies have revealed multiple mechanisms by which encounter with self antigens may abort B cell maturation and activation.

Central B Cell Tolerance

During their maturation in the bone marrow, B lymphocytes first express IgM as their antigen receptor and are functionally immature at this stage (see Chapter 8). If these B lymphocytes recognize self antigens in the bone marrow with high affinity, they either change their specificity or are deleted (Fig. 15.11).

- ***Receptor editing.*** If immature B cells recognize self antigens that are present at high concentration in the bone marrow, and especially if the antigen is displayed in multivalent form (e.g., on cell surfaces), many antigen receptors on each B cell are cross-linked, thus delivering strong signals to the cells. One consequence of such signaling is that the immature B cells reactivate their *RAG1* and *RAG2* genes and initiate a new round of VJ recombination in the Ig κ light chain gene locus (see Chapter 8). A Vκ segment upstream of the already rearranged VκJκ unit is joined to a downstream Jκ. As a result, the previously rearranged VκJκ exon in the self-reactive immature B cell is removed, and a new Ig light chain is expressed, thus creating a B cell receptor (BCR) with a new specificity. This process is called **receptor editing** and is an important mechanism for eliminating self-reactivity from the mature B cell repertoire. If the edited light chain rearrangement is nonproductive, additional Vκ-to-Jκ rearrangements will be made in the same locus, and if these fail, the process may proceed at the κ locus on the other chromosome; if that is nonproductive, rearrangements at the λ light chain loci may follow. A B cell expressing a λ light chain is likely a cell that has undergone receptor editing. It is estimated that among peripheral blood B cells in humans, as many as one-quarter to one-half of all the cells and the majority of λ-expressing cells have undergone receptor editing during their maturation.

- ***Deletion.*** Strong recognition of self antigens by immature/transitional B cells in the bone marrow or soon after these cells emerge from the bone marrow may lead to apoptosis of the B cells. This is presumably similar to apoptosis in T cells, when high-affinity antigen recognition in the thymus triggers the mitochondrial pathway of apoptosis, but the mechanisms are not well defined. It is likely that self-reactive B cells first try to change their specificity and die if receptor editing fails.



A
High-avidity self antigen recognition
Self antigen
Self-reactive B cell
Receptor editing: expression of new Ig light chain
Apoptosis
Non-self reactive B cell
Deletion
B
Low-avidity self antigen recognition
Self antigen
Reduced receptor expression, signaling
Anergic B cell

FIGURE 15.11 Central tolerance in B cells. **A,** Immature B cells that recognize self antigens in the bone marrow with high avidity (e.g., multivalent arrays of antigens on cells) die by apoptosis or change the specificity of their antigen receptors (receptor editing, which involves only light chains but is illustrated as a change in the antigen-binding region of the receptor). **B,** Weak recognition of self antigens in the bone marrow may lead to anergy (functional inactivation) of the B cells.

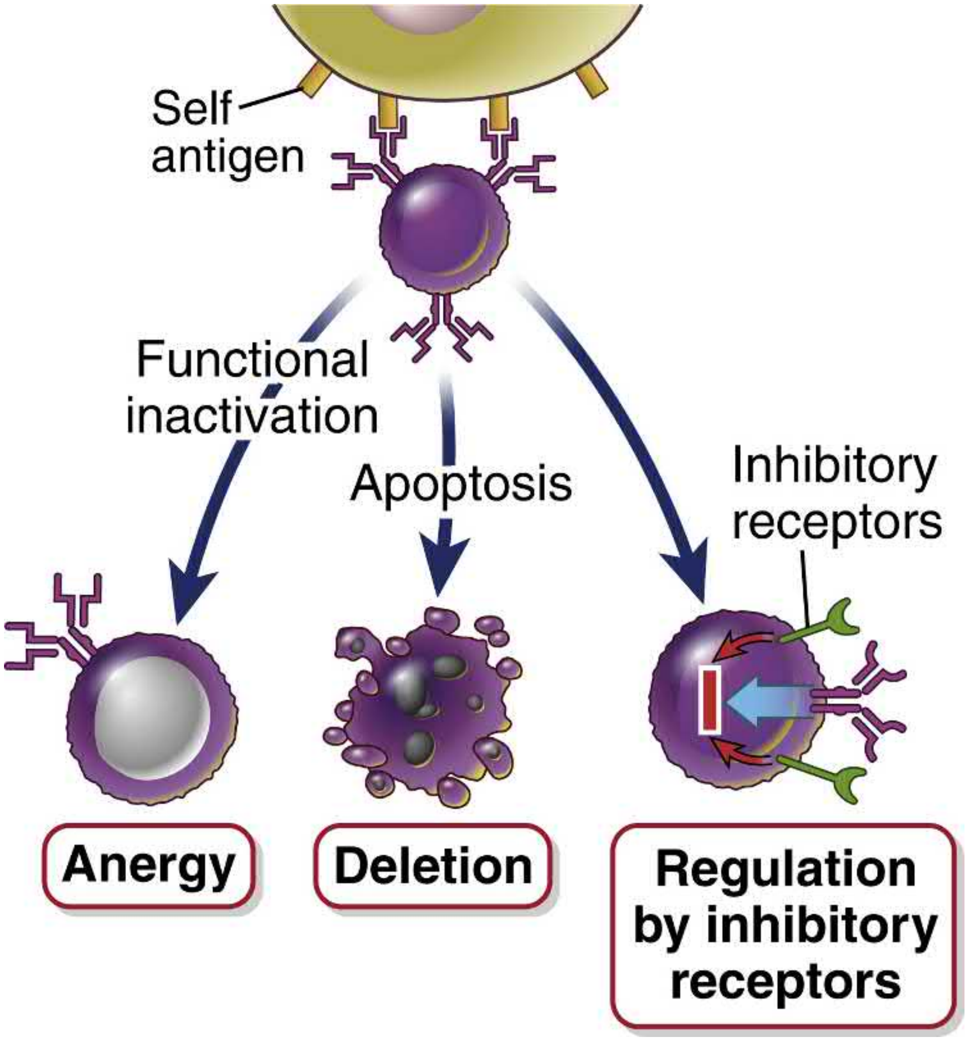

Self antigen
Functional inactivation
Apoptosis
Inhibitory receptors
Anergy
Deletion
Regulation by inhibitory receptors

FIGURE 15.12 Peripheral tolerance in B cells. B cells that encounter self antigens in peripheral tissues become anergic or die by apoptosis. In some situations, recognition of self antigens may trigger inhibitory receptors that prevent B cell activation.

- ***Anergy.*** If developing B cells recognize self antigens weakly (e.g., if the antigen is soluble and does not cross-link many antigen receptors or if the BCRs recognize the antigen with low affinity), the cells become functionally unresponsive (anergic) and exit the bone marrow in this unresponsive state. Anergy is due to downregulation of antigen receptor expression and a block in antigen receptor signaling.

Peripheral B Cell Tolerance

Mature B lymphocytes that recognize self antigens in peripheral tissues in the absence of specific helper T cells may be rendered functionally unresponsive or die by apoptosis (Fig. 15.12). Signals from helper T cells may be absent if these T cells are deleted or anergic (in the case of protein antigens) or if the self antigens are nonprotein antigens. Because self antigens do not elicit innate immune responses, B cells will also not be activated via complement receptors or pattern recognition receptors. Thus, as in T cells, antigen recognition without additional stimuli results in tolerance. Peripheral tolerance mechanisms also eliminate autoreactive B cell clones that may be generated as an unintended consequence of somatic mutation in germinal centers.

Several mechanisms of peripheral tolerance in B cells have been described.

- ***Anergy.*** Some self-reactive B cells that are repeatedly stimulated by self antigens become unresponsive to further activation. Anergic B cells require higher than normal levels of the growth factor BAFF (B-cell activating factor, also called BLys [B lymphocyte stimulator]) for survival, and they cannot compete with normal naive B cells for BAFF. As a result, the B cells that have encountered self antigens have a shortened life span and are eliminated more rapidly than cells that have not recognized self antigens.
- ***Deletion.*** B cells that bind with high avidity to self antigens in the periphery may also undergo apoptotic death by the mitochondrial pathway. Deletion of B cells specific for protein antigens may occur outside the bone marrow at the transitional B cell stages found in the spleen and the blood. Transitional B cells are cells that are in the process of maturing in the periphery into follicular B cells. A large fraction of transitional B cells may be autoreactive, and most of them are deleted and do not mature into follicular B cells. The mechanisms of deletion at the transitional B cell

stage are not well defined. Somatic mutation of Ig genes during the germinal center reaction may also lead to the emergence of self-reactive B cells (see Chapter 12). Some of these autoreactive B cells may be deleted by the FAS pathway.

- ***Signaling by inhibitory receptors.*** B cells that recognize self antigens may be prevented from responding by the engagement of various inhibitory receptors. The function of these inhibitory receptors is to set a threshold for B cell activation, which allows responses to foreign antigens because these typically elicit strong signals from the combination of BCR, coreceptors, innate immune receptors, and helper T cells (for protein antigens), but does not allow responses to self antigens, which engage only the BCR. This mechanism of peripheral tolerance was revealed by studies showing that mice with defects in the tyrosine phosphatase SHP1, the tyrosine kinase LYN, and the inhibitory receptors FcγRIIB and CD22 develop autoimmunity. CD22, also known as Siglec-2, binds to sialic acid that decorates sugar side chains of proteins on the B cell surface and dampens BCR signaling. Immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the cytoplasmic tail of CD22 are phosphorylated by LYN, and this inhibitory receptor then recruits SHP1, thus attenuating BCR signaling. How exactly CD22 contributes to B cell tolerance is not fully understood.
- ***Regulation of B cells by Tregs.*** A specialized subset of Tregs, called T follicular regulatory (Tfr) cells, enter lymphoid follicles and contribute to the attenuation of T cell help to germinal center B cells during antibody responses. Defects in regulatory T cells have also been shown to result in increased numbers of Tfh cells being generated outside the follicle at the boundary between the B cell zone and the T cell zone. Overall, Tregs outside the follicle and Tfr cells in the germinal center contribute together to limit Tfh cell numbers and activity and thus prevent promiscuous B cell

activation. Predictably, deficiency of Tregs is associated with autoantibody production.

Tolerance to Commensal Microbes and Other Foreign Antigens

Commensal microbes are abundant in the gut, skin, and other tissues but do not elicit immune responses despite being foreign. There are several reasons for this lack of immunogenicity. Many of these microbes cannot invade epithelial barriers and therefore may not be accessible to the adaptive immune system. These microbes also induce and activate Tregs, which prevent the development of effector and memory cells (see Chapter 14).

Foreign antigens may be administered in ways that preferentially induce tolerance rather than immune responses. In general, protein antigens administered with adjuvants favor immunity, whereas repeated doses of antigens administered without adjuvants tend to induce tolerance. The likely reason for this is that adjuvants stimulate innate immune responses and the expression of costimulators on APCs, and in the absence of these second signals, T cells that recognize the antigen may become anergic or die or may differentiate into Tregs. Many other features of antigens, and how they are administered, may influence the balance between immunity and tolerance (see Table 15.2).

The oral administration of a protein antigen often leads to suppression of systemic humoral and cell-mediated immune responses to immunization with the same antigen. This phenomenon, called **oral tolerance,** was discussed in Chapter 14.

Therapeutic Tolerance Induction

Understanding how to induce tolerance by antigen administration is the key to developing antigen-specific tolerance as a treatment strategy for immunologic diseases. Numerous clinical studies are under way to induce tolerance in autoimmune diseases and allergies in which the relevant antigen is known. The autoimmune diseases include type 1 diabetes (in which a major self antigen is insulin), MS (myelin basic protein), and rheumatoid arthritis (citrullinated peptides). Several strategies are being tried.

- Repeated administration of small doses of immunodominant peptides derived from the self antigen, in aqueous form without adjuvants. It is expected that the antigen will be recognized in the absence of costimulation and will induce peripheral T cell anergy or deletion or preferentially activate Tregs, but the cellular consequence of antigen administration remains unknown in these clinical studies. It is also unclear how effective this approach will be in inhibiting ongoing immune responses in patients. This approach has been used for many years to treat allergies (the process called desensitization or peptide immunotherapy, see Chapter 20). It is effective in a subset of allergic patients, but how desensitization works is also not established.
- Administration of peptides derived from self antigens coupled to nonimmunogenic substrates such as nanoparticles and erythrocyte membranes. The idea is similar to aqueous antigen administration, described previously.
- Transfer or activation of Tregs has been mentioned previously.
- Costimulatory blockade refers to blocking B7 molecules using the drug CTLA4-Ig (see Chapter 9). It is likely this treatment inhibits immune responses but does not induce long-lived tolerance.

Despite the promise of these approaches, there has so far been little success in preventing immune responses against autoantigens in patients with ongoing autoimmune diseases. Defining the mechanisms by which the different strategies may be working will require analysis of antigen-specific lymphocytes, for which the technology is still developing.

Mechanisms of Autoimmunity

The possibility that an individual's immune system may react against autologous antigens and cause tissue injury was appreciated by immunologists from the time that the specificity of the immune system for foreign antigens was recognized. In the early 1900s, Paul Ehrlich coined the rather melodramatic phrase *horror autotoxicus* (the horror of self-toxicity) to describe the body's fear of self-destruction by the immune system. Autoimmunity is an important cause of disease in humans, estimated to affect at least 2% to 5% of the U.S. population, and the incidence of many autoimmune diseases seems to be rising. The term autoimmunity is often erroneously used for any disease in which immune reactions accompany tissue injury, even though it may be difficult or impossible to establish a role for immune responses against particular self antigens in causing these disorders. Because inflammation is a prominent component of these disorders, they are sometimes grouped under immune-mediated inflammatory diseases, which does not imply that the pathologic response is directed against self antigens (see Chapter 19).

The fundamental questions about autoimmunity are how self-tolerance fails and how self-reactive lymphocytes are activated. Answers to these questions are needed to understand the cause and pathogenesis of autoimmune diseases, which is a major challenge in immunology. Our understanding of autoimmunity has improved greatly during the past two decades, mainly because of the development of informative animal models of these diseases, the identification of genes that may predispose to autoimmunity, and improved methods for analyzing immune responses in humans.

The factors that contribute to the development of autoimmunity are genetic susceptibility and environmental triggers, such as infections and local tissue injury. Susceptibility genes may disrupt self-tolerance mechanisms, and infection or necrosis in tissues promotes the influx of autoreactive lymphocytes and activation of these cells, resulting in tissue injury (Fig. 15.13). Infections and tissue injury may also alter the way in which self antigens are displayed to the immune system and induce production of pro-inflammatory cytokines, leading to failure of self-tolerance and activation of self-reactive lymphocytes. The roles of these factors in the development of autoimmunity are discussed later. Other factors such as changes in the host microbiome may play roles in pathogenesis and are being actively studied, but this field of inquiry is still in its infancy.

General Features of Autoimmune Disorders

Autoimmune diseases may be systemic or organ specific, depending on the distribution of the autoantigens that are recognized. For example, the formation of circulating immune complexes composed of self antigens and specific antibodies typically produces systemic diseases, such as systemic lupus erythematosus (SLE). In contrast, autoantibody and T cell responses against self antigens with restricted tissue distribution lead to organ-specific diseases, such as myasthenia gravis affecting muscle function, type 1 diabetes affecting insulin production by the pancreas, and MS affecting myelinated nerve function.

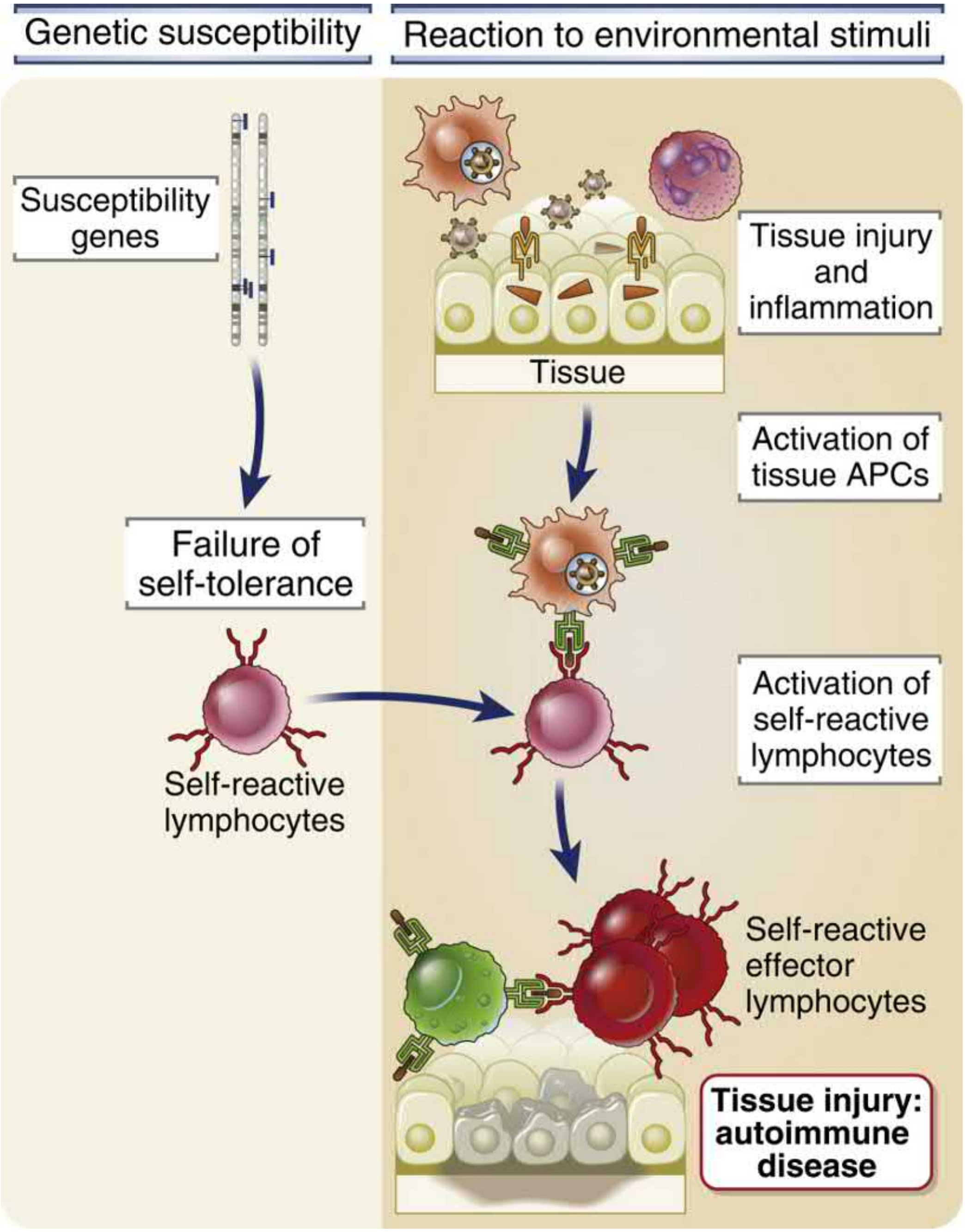

Genetic susceptibility
Reaction to environmental stimuli
Susceptibility genes
Tissue injury and inflammation
Tissue
Activation of tissue APCs
Failure of self-tolerance
Activation of self-reactive lymphocytes
Self-reactive lymphocytes
Self-reactive effector lymphocytes
Tissue injury: autoimmune disease

FIGURE 15.13 Postulated mechanisms of autoimmunity.In this proposed model of an organ-specific T cell–mediated autoimmune disease, various genetic loci may confer susceptibility to autoimmunity, in part by influencing the maintenance of self-tolerance. Environmental triggers, such as infections and other

inflammatory stimuli, promote the influx of lymphocytes into tissues and the activation of self-reactive T cells, resulting in tissue injury. *APCs,* Antigen-presenting cells.

Various effector mechanisms are responsible for tissue injury in different autoimmune diseases. These mechanisms include immune complexes, circulating autoantibodies, and autoreactive T lymphocytes and are discussed in Chapter 19. The clinical and pathologic features of the disease are usually determined by the nature of the dominant autoimmune response.

Autoimmune diseases tend to be chronic, progressive, and self-perpetuating. The reasons for these features are that the self antigens that trigger these reactions are persistent, and once an immune response starts, many amplification mechanisms are activated that perpetuate the response. In addition, a response initiated against one self antigen that injures tissues may result in the release and alterations of other tissue antigens, activation of lymphocytes specific for these other antigens, and exacerbation of the disease. This phenomenon is called **epitope spreading,** and it may explain why once an autoimmune disease has developed, it may become prolonged and self-perpetuating.

Immunologic Abnormalities Leading to Autoimmunity

Several immunologic aberrations have been associated with the development of autoimmunity in humans and experimental models. The main such abnormalities are the following:

- ***Defective self-tolerance. Inadequate elimination or regulation of T or B cells, leading to an imbalance between lymphocyte activation and control, is the underlying cause of all autoimmune diseases.*** The potential for autoimmunity exists in all individuals because some of the clones of developing

lymphocytes may express receptors specific for self antigens, and not all of these clones are deleted during their maturation, so many self-reactive lymphocytes are present in healthy individuals. Also, many self antigens are readily accessible to lymphocytes. As discussed earlier, tolerance to self antigens is normally maintained by selection processes that prevent the maturation of some self antigen–specific lymphocytes and by mechanisms that inactivate or delete self-reactive lymphocytes that do mature. Loss of self-tolerance may result if self-reactive lymphocytes are not deleted or inactivated and if APCs are activated so that self antigens are presented to the immune system in an immunogenic manner. Experimental models and some results from analyses of human diseases have shown that any of the following mechanisms may contribute to the failure of self-tolerance:
  - ○ Defects in deletion (negative selection) of T or B cells or receptor editing in B cells during the maturation of these cells in the generative lymphoid organs
  - ○ Defective numbers or functions of regulatory T lymphocytes
  - ○ Defective apoptosis of mature self-reactive lymphocytes
  - ○ Inadequate function of inhibitory receptors
- ***Abnormal display of self antigens.*** Abnormalities may include structural changes in self antigens resulting from enzymatic modifications or from cellular stress or injury. If these changes lead to the display of antigenic epitopes that are not present normally, the immune system may not be tolerant to these “neoantigens,” thus allowing anti-self responses to develop. Examples include citrullination of various self peptides in rheumatoid arthritis and post-translational modifications of islet proteins in type 1 diabetes.
- ***Inflammation or an initial innate immune response.*** As we have discussed in previous chapters, the innate immune response is a strong stimulus for the activation of

lymphocytes and the generation of adaptive immune responses. Infections or cell injury may elicit local innate immune reactions with inflammation. These may contribute to the development of autoimmune disease, perhaps by activating APCs to express increased levels of costimulators, which overcome regulatory mechanisms and result in excessive T cell activation. Cytokines made during innate immune responses are also capable of activating T and B lymphocytes. An example is the production of type I IFN associated with SLE and other autoimmune diseases.

Much recent attention has focused on the role of T cells in autoimmunity for two main reasons. First, helper T cells are the key regulators of both cell-mediated and humoral immune responses to proteins, and most self antigens implicated in autoimmune diseases are proteins. Second, the risk for developing several autoimmune diseases is associated with the inheritance of certain MHC alleles (the HLA [human leukocyte antigen] complex in humans), and the function of MHC molecules is to present peptide antigens to T cells. Failure of self-tolerance in T lymphocytes may result in autoimmune diseases in which tissue damage is caused by cell-mediated immune reactions. Helper T cell abnormalities may also lead to autoantibody production because helper T cells are necessary for the production of high-affinity antibodies against protein antigens.

In the following section, we describe the general principles of the pathogenesis of autoimmune diseases, with an emphasis on susceptibility genes, infections, and other factors that contribute to the development of autoimmunity. We will describe the pathogenesis and features of some illustrative autoimmune diseases in Chapter 19.

Genetic Basis of Autoimmunity

From the earliest studies of autoimmune diseases in patients and experimental animals, it has been appreciated that these diseases have a strong genetic component. For example, type 1 diabetes shows a concordance of 35% to 50% in monozygotic twins and 5% to 6% in dizygotic twins, and other autoimmune diseases show similar evidence of a genetic contribution. Linkage analyses in families, genome-wide association studies, and large-scale sequencing efforts are revealing new information about the genes that may play causal roles in the development of autoimmunity and chronic inflammatory disorders.

Most autoimmune diseases are complex polygenic traits in which affected individuals inherit multiple genetic polymorphisms that contribute to disease susceptibility, and these genes act with environmental factors to cause the diseases. Some of these polymorphisms are associated with several autoimmune diseases, suggesting that the associated genes influence general mechanisms of immune regulation and self-tolerance. Other loci are associated with particular diseases, suggesting that they may affect organ damage or autoreactive lymphocytes of particular specificities. Each genetic polymorphism makes a small contribution to the development of particular autoimmune diseases and is also found in healthy individuals but at a lower frequency than in patients with the diseases. It is postulated that in individual patients, multiple such polymorphisms are coinherited and together account for development of the disease. Understanding the interplay of multiple genes with one another and with environmental factors is one of the continuing challenges in the field.

The best-characterized genes associated with autoimmune diseases and our current understanding of how they may contribute to loss of self-tolerance are described here.

Association of MHC Alleles With Autoimmunity

Among the genes that are associated with autoimmunity, the strongest associations are with MHC genes. In fact, in many autoimmune diseases, such as type 1 diabetes, 20 or 30 disease-associated genes have been identified; in most of these diseases, the HLA locus alone contributes half or more of the genetic susceptibility. HLA typing of large groups of patients with various autoimmune diseases has shown that some HLA alleles occur at higher frequency in these patients than in the general population. From such studies, one can calculate the odds ratio for development of a disease in individuals who inherit various HLA alleles (often referred to as the relative risk) (Table 15.3). The strongest such association is between ankylosing spondylitis, an inflammatory and presumably autoimmune disease of vertebral joints, and the class I HLA allele B27. Individuals who are HLA-B27 positive are over 100 times more likely to develop ankylosing spondylitis than individuals who are B27-negative. Neither the mechanism of this disease nor the basis of its association with HLA-B27 is known. The association of class II HLA-DR and HLA-DQ alleles with autoimmune diseases has received great attention, mainly because class II MHC genes are more frequently associated with autoimmune diseases (as well other immune-mediated disorders) than are class I MHC genes. Class II MHC molecules are involved in the selection and activation of $CD4^+$ T cells, and $CD4^+$ T cells regulate all immune responses to protein antigens.

Table 15.3

Disease	HLA Allele	Odds Ratio[a]
RA (anti-CCP Ab positive)[b]	*DRB1, 1 SE* allele[c]	4
	***DRB1, 2* SE alleles**	12
T1D	*DRB1 ∗ 0301-DQA1 ∗ 0501-DQB1 ∗ 0201* haplotype	4
	***DRB1 ∗ 0401-DQA1 ∗ 0301-DQB1 ∗0302* haplotype**	8
	***DRB1 ∗ 0301/0401* heterozygotes**	35
Multiple sclerosis	*DRB1 ∗ 1501*	3
SLE	*DRB1 ∗ 0301*	2
	DRB1 ∗ 1501	1.3
AS	*B27* (mainly *B ∗ 2705* and *B ∗ 2702*)	100
Celiac disease[d]	*DQA1 ∗ 0501-DQB1 ∗ 0201* haplotype	7

AS, Ankylosing spondylitis; *RA,* rheumatoid arthritis; *SLE,* systemic lupus erythematosus; *T1D,* type 1 diabetes.

[a] The odds ratio approximates values of increased risk for the disease associated with inheritance of particular HLA alleles. The data are from populations of European ancestry. Alleles of individual major histocompatibility complex genes (e.g., *DRB1*) are indicated by four numbers (e.g., 0301), based on serologic and molecular typing.

[b] Anti-CCP Ab, antibodies directed against cyclic citrullinated peptides. Data are from patients who test positive for these antibodies in the serum.

[c] SE refers to shared epitope, so called because it is a consensus sequence in the DRB1 protein (positions 70–74) present in multiple *DRB1* alleles.

[d] Celiac disease is an immunologic reaction to gliadin, a protein in gluten, and not an autoimmune disease.

Courtesy Dr. Michelle Fernando, Imperial College, London.

Several features of the association of HLA alleles with autoimmune diseases are noteworthy.

- An HLA-disease association may be identified by serologic typing of one HLA locus, but the actual association may be with other alleles that are linked to the typed allele and inherited together. For example, individuals with a particular HLA-DR allele (hypothetically DR3) may show a higher probability of inheriting a particular HLA-DQ allele (hypothetically DQ5) than the probability of inheriting these alleles separately and randomly (i.e., at equilibrium) in the population. Such inheritance is an example of linkage disequilibrium. A disease may be found to be DR3 associated by HLA typing, but the causal association may actually be with the coinherited DQ5. This realization has emphasized the concept of extended HLA haplotypes, which refers to sets of linked genes, both classical HLA and adjacent non-HLA genes, that tend to be inherited together as a single unit.

- In many autoimmune diseases, the disease-associated nucleotide polymorphisms encode amino acids in the peptide-binding clefts of the MHC molecules. This observation is not surprising because polymorphic residues of MHC molecules are located within and adjacent to the clefts, and the structure of the clefts is the key determinant of both functions of MHC molecules, namely, antigen presentation and recognition by T cells (see Chapter 6).
- Disease-associated HLA sequences are found in healthy individuals. In fact, if all individuals bearing a particular disease-associated HLA allele are monitored prospectively, most will never develop the disease. Therefore, expression of a particular HLA gene is not by itself the cause or predictor of any autoimmune disease, but it may be one of several factors that contribute to autoimmunity.
- Inheritance of certain HLA alleles confers protection against developing some autoimmune diseases, such as type 1 diabetes and rheumatoid arthritis.

The mechanisms underlying the association of different HLA alleles with various autoimmune diseases are still not clear. In diseases in which particular MHC alleles increase the risk, the disease-associated MHC molecule may present a self peptide and activate pathogenic T cells, and this has been established in a few cases. But a key question has not been answered in most cases: whether a disease-associated HLA molecule presents the relevant self antigen differently from HLA molecules that are not associated with the disease. When a particular allele is shown to be protective, it is hypothesized that this allele might induce negative selection of some potentially pathogenic T cells, or it might promote the development of Tregs, but there is no clear evidence in support of either hypothesis.

Polymorphisms in Non-HLA Genes Associated With Autoimmunity

The technique of genome-wide association studies has led to the putative identification of nucleotide polymorphisms (variants) of several genes that are associated with autoimmune diseases, and this has been extended by more recent genome sequencing efforts. Before the genes that are most clearly validated are discussed, it is important to summarize some of the general features of these genes.

- As stated earlier, it is likely that combinations of multiple inherited genetic polymorphisms interacting with environmental factors induce the immunologic abnormalities that lead to autoimmunity.
- Many of the polymorphisms associated with various autoimmune diseases are in genes that influence the development and regulation of immune responses. Although this conclusion appears predictable, it has reinforced the usefulness of the approaches being used to identify autoimmunity-associated genes.
- Different polymorphisms may protect against disease development or increase the incidence of the disease. The statistical methods used for genome-wide association studies have revealed both types of associations.
- Most disease-associated polymorphisms are located in noncoding regions of genes. This suggests that many of the polymorphisms may affect the expression of the encoded proteins.

Some of the many genes associated with human autoimmune diseases, which have been defined by linkage analyses, genome-wide association studies, and whole genome sequencing, are listed in Table 15.4, and a few are briefly described next.

- ***PTPN22.*** A variant of the protein tyrosine phosphatase PTPN22, in which arginine at position 620 is replaced with a tryptophan, is associated with rheumatoid arthritis, type 1 diabetes, autoimmune thyroiditis, and other autoimmune diseases. The disease-associated variant causes complex signaling alterations in multiple immune cell populations. Precisely how these changes lead to autoimmunity is not known.
- ***NOD2.*** Polymorphisms in this gene are associated with Crohn's disease, one type of IBD. NOD2 is a cytoplasmic sensor of bacterial peptidoglycans (see Chapter 4) and is expressed in multiple cell types, including intestinal epithelial cells. It is postulated that the disease-associated polymorphism reduces the function of NOD2, so it cannot provide effective defense against certain intestinal microbes. As a result, these microbes are able to traverse the epithelium and initiate a chronic inflammatory reaction in the intestinal wall, which is a hallmark of IBD (see Chapter 19). Crohn's disease may be an unregulated response to commensal microbes that has features of autoimmune diseases.
- ***Complement proteins.*** Genetic deficiencies of several complement proteins, including C1q, C2, and C4 (see Chapter 13), are associated with SLE. The postulated mechanism of this association is that complement activation promotes the clearance of circulating immune complexes and apoptotic cell bodies, and in the absence of complement proteins, these complexes accumulate in the blood and are deposited in tissues and the antigens of dead cells persist. There is also some evidence that complement activation increases signaling in B cells and promotes tolerance, but how or even if the complement system is activated by self antigens is unclear.

- ***IL-23 receptor (IL-23R).*** Some polymorphisms in the receptor for IL-23 are associated with increased susceptibility to IBD and the skin disease psoriasis, whereas other polymorphisms protect against development of these diseases. IL-23 is one of the cytokines involved in the development of Th17 cells, which stimulate inflammatory reactions (see Chapter 10).
- ***CD25 (IL-2Rα).*** Polymorphisms affecting the expression or function of CD25, the α chain of the IL-2 receptor, are associated with MS, type 1 diabetes, and other autoimmune diseases. These changes in CD25 likely affect the generation or function of Tregs, although there is no definitive evidence for a causal link between the CD25 abnormality, Treg defects, and the autoimmune disease.
- ***Fc γ RIIB.*** A polymorphism altering an isoleucine to a threonine in the transmembrane domain of this inhibitory Fc receptor impairs inhibitory signaling and is associated with SLE in humans. Genetic deletion of this receptor in mice also results in a lupus-like autoimmune disease. The likely mechanism of the disease is defective antibody-mediated feedback inhibition of B cells (see Chapter 12).
- ***ATG16L1.*** A loss-of-function polymorphism in this gene, which replaces a threonine in position 300 with an alanine, is associated with Crohn's disease. ATG16L1 is one of a family of proteins involved in autophagy, a cellular response to infection, nutrient deprivation, and other forms of stress. How this polymorphism contributes to IBD is not known; some possible mechanisms are discussed in Chapter 19.
- ***Insulin.*** Polymorphisms in the insulin gene that encode variable numbers of repeat sequences are associated with type 1 diabetes. These polymorphisms may affect the thymic expression of insulin. It is postulated that if the protein is expressed at low levels in the thymus because of a genetic polymorphism, developing T cells specific for insulin may not be negatively selected. These cells survive

in the mature immune repertoire and are capable of attacking insulin-producing islet β cells and causing diabetes.

Although many genetic associations with autoimmune diseases have been reported, a continuing challenge is to correlate the genetic polymorphisms with the pathogenesis of the diseases.

Inherited Single-Gene (Mendelian) Abnormalities That Cause Autoimmunity

Studies with mouse models and patients have identified several genes that strongly influence the maintenance of tolerance to self antigens (Table 15.5). Unlike the complex polymorphisms described previously, these single-gene defects are examples of Mendelian disorders in which the mutation is rare but has a high penetrance, so that most individuals carrying the mutation are affected. We mentioned many of these genes earlier in the chapter, when we discussed the mechanisms of self-tolerance. Although these genes are associated with rare autoimmune diseases, their identification has provided valuable information about the importance of various mechanisms in the maintenance of self-tolerance. The proteins encoded by these genes contribute to central tolerance *(AIRE)*, generation and function of Tregs *(FOXP3, IL2, IL2R)*, anergy and the function of Tregs *(CTLA4)*, peripheral deletion of T and B lymphocytes *(FAS, FASL)*, and regulation of pathogenic T cells in mucosal tissues *(IL10, IL10R)*. None of these mutations is seen in common autoimmune diseases.

Table 15.4

Gene (Encoded Protein)	**Function of Protein**	**Disease**
Signaling and Transcription Factors		
PTPN22	TCR and BCR signaling and other?	RA, SLE, AITD, T1D
BLK	B cell activation	SLE
IRF5	Type I IFN production	SLE
TRAF1	Regulates TNFR signaling, NF-κB pathway	RA
STAT4	IFN-γ response	RA, SLE
Innate Immunity		
NOD2	Cytosolic receptor for bacterial peptidoglycans	CD
***C1QA*, C2, C4** **(Complement C1q, C2, C4)**	Clearance of immune complexes and apoptotic bodies; role in B cell tolerance?	SLE
Cytokines, Cytokine Receptors, Cytokine Signaling		
IL2	T cell activation, Treg maintenance (IL-2)	T1D, RA, celiac disease
IL23R **(IL-23 receptor)**	Th17 differentiation	PSA, PSO, CD, AS
***IL2RA* (CD25)**	T cell activation, Treg maintenance	MS, T1D, GD
***IL7RA* (CD127)**	Survival of naive and memory T cells	MS
***IL12B* (p40)**	Th1 differentiation	PSO, CD
IL10	Inhibition of Th1 responses	IBD, SLE, T1D
Lymphocyte Regulation		
CTLA4	T cell inhibition, Treg function	T1D, RA
***FCGR2B* (FcγRIIB)**	Feedback inhibition of B cells	SLE
Autophagy Related		
ATG16L1	Autophagy	CD
Autoantigens		
***INS* (insulin)**	Islet β cell antigen	T1D
TSHR **(thyroid-stimulating hormone receptor)**	Thyroid antigen	AITD
Antigen Processing or Modifying Enzymes		
ARTS1	Peptide trimming for class I MHC pathway	AS
PAD14	Citrullination of self peptides	RA

The table lists some of the non-HLA gene loci in which polymorphisms are associated with various common autoimmune diseases. The encoded proteins are indicated when the gene name is not identical to the protein. Selected examples are discussed in the text.

AITD, Autoimmune thyroid disease; *AS,* ankylosing spondylitis; *BCRs,* B cell receptors; *CD,* Crohn's disease; *GD,* Graves' disease; *IL,* interleukin; *MHC,* major histocompatibility complex; *MS,* multiple sclerosis; *PSA,* psoriatic arthritis; *PSO,* psoriasis; *RA,* rheumatoid arthritis; *SLE,* systemic lupus erythematosus; *T1D,* type 1 diabetes; *TCRs,* T cell receptors.

Modified from Gregersen PK, Olsson LM. Recent advances in the genetics of autoimmune disease. *Annu Rev Immunol*. 2009;27:363–391.

Table 15.5

Gene	**Phenotype of Mutant or Knockout Mouse**	**Mechanism of Failure of Tolerance**	**Human Disease**
AIRE	Destruction of endocrine organs by antibodies, lymphocytes	Failure of central tolerance	APS
C4	SLE	Defective clearance of immune complexes, failure of B cell tolerance	SLE
CTLA4	Lymphoproliferation; T cell infiltrates in multiple organs; lethal by 3–4 wk	Defective function of Tregs, failure of T cell anergy	Systemic inflammatory disease
FAS/FASL	Anti-DNA and other autoantibodies, immune complex nephritis, arthritis, lymphoproliferation	Defective deletion of self-reactive B cells and CD4$^+$ T cells	ALPS
FOXP3	Multiorgan lymphocytic infiltrates, wasting	Deficiency of functional Tregs	IPEX
IL10, IL10R	Inflammatory bowel disease	Defective control of mucosal immune responses	Colitis (IL10R mutations)
IL2RA/B, IL2	Inflammatory bowel disease; anti-erythrocyte and anti-DNA autoantibodies	Defective development, survival, or function of Tregs	Multisystem autoimmune disease
SHP1	Multiple autoantibodies	Failure of negative regulation of B cells	None known

The roles of these mutations in causing autoimmunity have been established by inherited diseases in humans and gene knockouts in mice.

AIRE, Autoimmune regulator gene; *ALPS,* autoimmune lymphoproliferative syndrome; *APS,* autoimmune polyendocrine syndrome; *IL-2,* interleukin-2; *IPEX,* immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; *SHP-1,* SH2-containing phosphatase 1; *SLE,* systemic lupus erythematosus; *Tregs*, regulatory T cells.

Role of Infections in Autoimmunity

Viral and bacterial infections may contribute to the development and exacerbation of autoimmunity. In patients and in some animal models, the onset of autoimmune diseases is often associated with or preceded by infections. In most of these cases, the infectious microorganism is not present in lesions and is not even detectable in the individual when autoimmunity develops. Therefore, the lesions of autoimmunity are not due to the infection itself but result from host immune responses that may be triggered or dysregulated by the microbe.

Infections may promote the development of autoimmunity by two principal mechanisms (Fig. 15.14).

- Infections of particular tissues may induce local innate immune responses that recruit leukocytes into the tissues and result in the activation of tissue APCs. These APCs begin to express costimulators and secrete T cell–activating cytokines, resulting in the breakdown of T cell tolerance. Thus, the infection results in the activation of T cells that are specific for self antigens and not only for the infectious pathogen. The importance of aberrant expression of costimulators is suggested by experimental evidence that immunization of mice with self antigens together with strong adjuvants (which mimic microbes) results in the breakdown of self-tolerance and the development of autoimmune disease. In other experimental models, viral antigens expressed in tissues such as islet β cells induce T cell tolerance, but systemic infection of the mice with the virus results in the failure of tolerance and autoimmune destruction of the insulin-producing cells. Microbes may also engage Toll-like receptors (TLRs) on DCs, leading to the production of costimulators and lymphocyte-activating cytokines, and on autoreactive B cells, leading to autoantibody production. A role of TLR signaling in autoimmunity has been demonstrated in mouse models of lupus.

- Infectious microbes may contain antigens that cross-react with self antigens, so immune responses to the microbes may result in reactions against self antigens. This phenomenon is called **molecular mimicry** because the antigens of the microbe cross-react with, or mimic, self antigens. One example of an immunologic cross-reaction between microbial and self antigens is rheumatic fever, which develops after streptococcal infections and is caused by anti-streptococcal antibodies that cross-react with myocardial proteins. These antibodies are deposited in the heart and cause myocarditis. DNA sequencing has revealed numerous short stretches of homologies between myocardial proteins and streptococcal proteins. However, the significance of limited homologies between microbial and self antigens in common autoimmune diseases remains to be established.

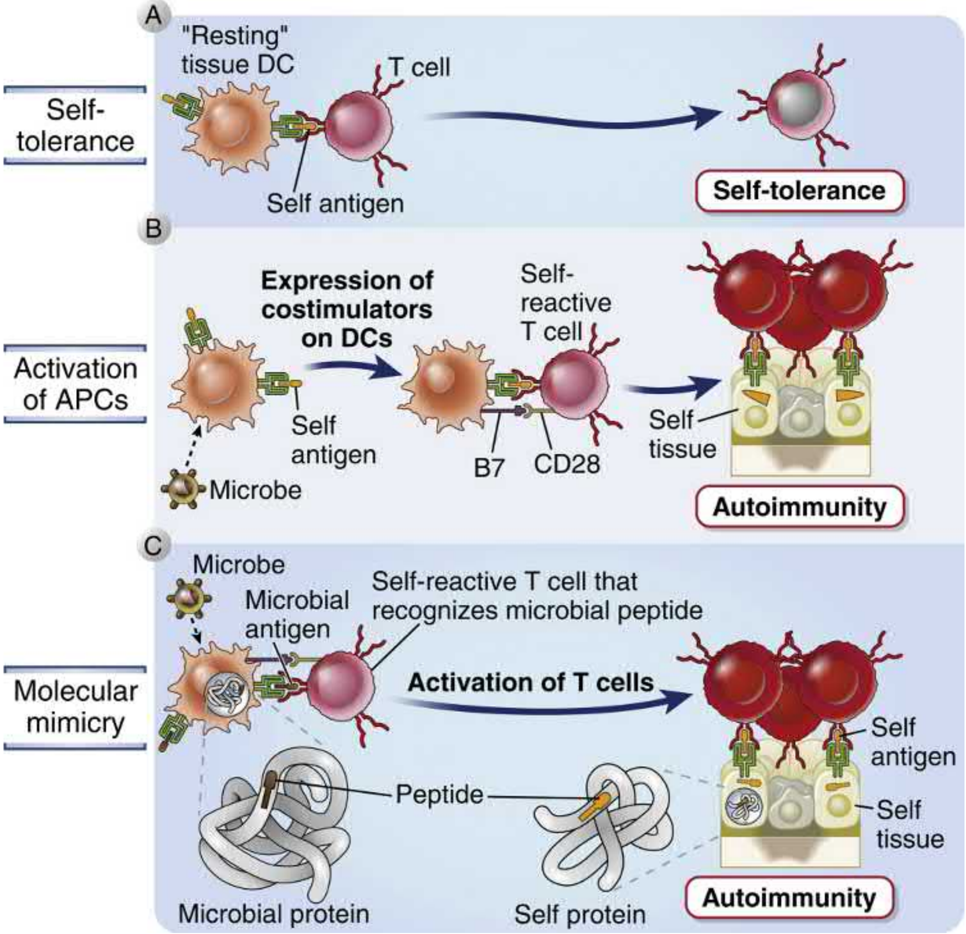

A
"Resting" tissue DC
T cell
Self-tolerance
Self antigen
Self-tolerance
B
Activation of APCs
Expression of costimulators on DCs
Self-reactive T cell
Self antigen
Microbe
B7
CD28
Self tissue
Autoimmunity
C
Molecular mimicry
Microbe
Microbial antigen
Self-reactive T cell that recognizes microbial peptide
Activation of T cells
Self antigen
Peptide
Self tissue
Microbial protein
Self protein
Autoimmunity

FIGURE 15.14 Role of infections in the development of autoimmunity. **A,** Normally, encounter of a mature self-reactive T cell with a self antigen presented by a costimulator-deficient resting tissue antigen-presenting cells *(APCs)* results in peripheral tolerance by anergy. (Other possible mechanisms of self-tolerance are not shown.) **B,** Microbes may activate the APCs to express costimulators, and when these APCs present self antigens the self-reactive T cells are activated rather than rendered tolerant. **C,** Some microbial antigens may cross-react with self antigens (molecular mimicry). Therefore, immune responses initiated by the microbes may

activate T cells specific for self antigens.
DCs, Dendritic cells.

Some infections may protect against the development of autoimmunity. Epidemiologic studies suggest that reducing infections increases the incidence of type 1 diabetes and MS, and experimental studies show that diabetes in nonobese diabetic mice is greatly retarded if the mice are infected. It seems paradoxical that infections can be triggers of autoimmunity and also inhibit autoimmune diseases. How they may reduce the incidence of autoimmune diseases is unknown.

The intestinal and cutaneous microbiome may influence the development of autoimmune diseases. As we discussed in Chapter 14, humans are colonized by commensal microbes that may have significant effects on the maturation and activation of the immune system. This idea is supported by the finding that alterations in the microbiome affect the incidence and severity of autoimmune diseases in experimental models. It is less clear if changes in the human microbiome affect the incidence of autoimmune diseases, mainly because of inherent variations and complexity in the microbiomes of individuals.

Other Factors in Autoimmunity

The development of autoimmunity is related to several factors in addition to susceptibility genes and infections.

- ***Anatomic alterations in tissues, caused by inflammation (possibly secondary to infections), ischemic injury, or trauma, may lead to the exposure of self antigens that are normally concealed from the immune system.*** Such sequestered antigens may not have induced self-tolerance. Therefore, if previously hidden self antigens are released, they can interact with immunocompetent lymphocytes and induce specific immune responses. Examples of anatomically sequestered antigens in so-called immune-privileged tissues include intraocular and testicular proteins (see Chapter 14). Post-traumatic uveitis and orchitis, which can be bilateral even when the trauma is unilateral, are thought to be due to autoimmune responses against self antigens that are released from their normal locations by trauma.

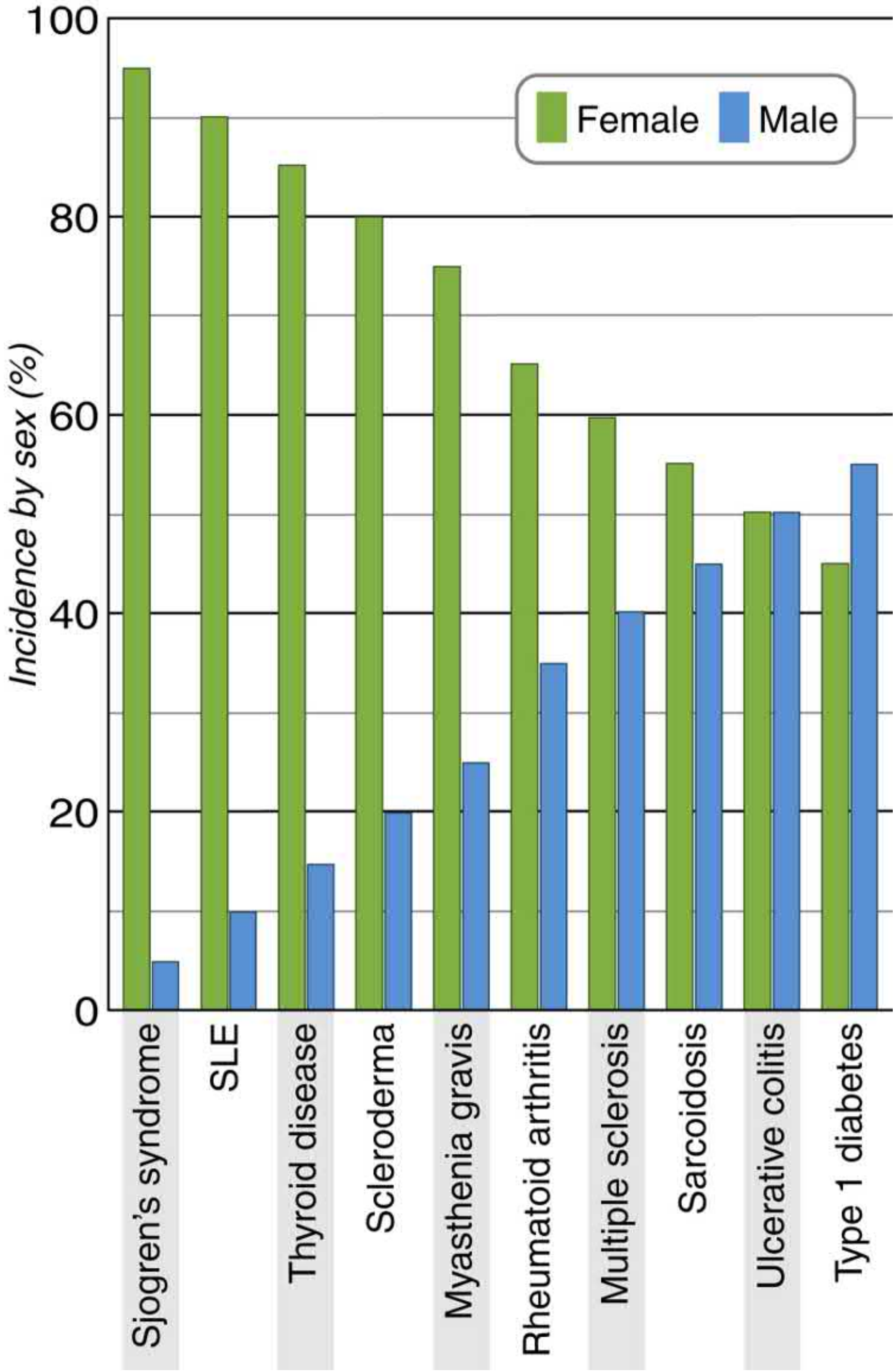


FIGURE 15.15 Gender distribution of major autoimmune diseases. The percentages are approximations based on incidence data until the year 2000. Included is an inflammatory

disease (sarcoidosis) that is not thought to be autoimmune. *SLE,* Systemic lupus erythematosus.

From Whitacre CC. Sex differences in autoimmune diseases. *Nat Immunol.* 2001;2:777. With permission of the publishers.

- ***Gender plays a role in several autoimmune diseases.*** Many autoimmune diseases have a higher incidence in women than in men (Fig. 15.15). For example, SLE affects women about 10 times more frequently than men. The lupus-like disease of (NZB × NZW)F_1 mice develops only in females and is retarded by androgen treatment. Whether this female predominance results from the influence of sex hormones or other gender-related factors is not known.

Autoimmune diseases are among the most difficult scientific and clinical problems in immunology. The current knowledge of pathogenic mechanisms remains incomplete, so theories and hypotheses continue to outnumber facts. The application of new technical advances and the rapidly improving understanding of self-tolerance will, it is hoped, lead to clearer and more definitive answers to the enigmas of autoimmunity.

Summary

- Immunologic tolerance is unresponsiveness to an antigen induced by the exposure of specific lymphocytes to that antigen. Tolerance to self antigens is a fundamental property of the normal immune system, and the failure of self-tolerance leads to autoimmune diseases. Antigens may be administered in ways that induce tolerance rather than immunity, and this may be exploited for the prevention and treatment of transplant rejection and autoimmune and allergic diseases.

- Central tolerance is induced in the generative lymphoid organs (thymus and bone marrow) when immature lymphocytes encounter self antigens present in these organs. Peripheral tolerance occurs when mature lymphocytes recognize self antigens in peripheral tissues under particular conditions.
- In T lymphocytes, central tolerance occurs when immature thymocytes with high-affinity receptors for self antigens recognize these antigens in the thymus. Some immature T cells that encounter self antigens in the thymus die (negative selection), and others develop into FOXP3$^+$ regulatory T lymphocytes (Tregs) that function to control responses to self antigens in peripheral tissues.
- Several mechanisms account for peripheral tolerance in mature T cells. In CD4$^+$ T cells, anergy is induced by antigen recognition without adequate costimulation or by engagement of inhibitory receptors such as CTLA-4 (cytotoxic T lymphocyte antigen 4) and PD-1 (programmed cell death protein-1). Tregs inhibit immune responses by multiple mechanisms. T cells that encounter self antigens without other stimuli or that are repeatedly stimulated may die by apoptosis.
- In B lymphocytes, central tolerance is induced when immature B cells recognize multivalent self antigens in the bone marrow. The result is the acquisition of a new specificity, called receptor editing, or apoptotic death of the immature B cells. Mature B cells that recognize self antigens in the periphery in the absence of T cell help may be rendered anergic and ultimately die by apoptosis or become functionally unresponsive because of the engagement of inhibitory receptors.
- Autoimmunity results from inadequate self-tolerance or regulation of lymphocytes. Autoimmune reactions may be triggered by environmental stimuli, such as infections, in genetically susceptible individuals.

- Most autoimmune diseases are polygenic, and numerous susceptibility genes contribute to disease development. The greatest contribution is from major histocompatibility complex (MHC) genes; other genes are thought to influence the selection or regulation of self-reactive lymphocytes.
- Infections may predispose to autoimmunity by several mechanisms, including enhanced expression of costimulators in tissues and cross reactions between microbial antigens and self antigens. Some infections may protect individuals from autoimmunity, by unknown mechanisms.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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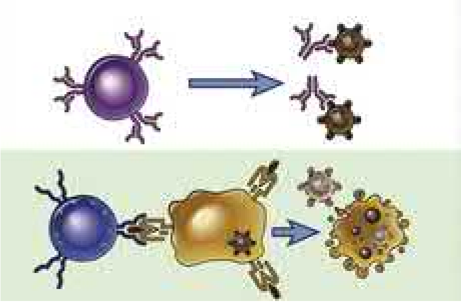
Chapter 16: Immunity to Microbes



- Overview of Immune Responses to Microbes,
- Immunity to Extracellular Bacteria,
  - Innate Immunity to Extracellular Bacteria,
  - Adaptive Immunity to Extracellular Bacteria,
  - Injurious Effects of Immune Responses to Extracellular Bacteria,
  - Immune Evasion by Extracellular Bacteria,
- Immunity to Intracellular Bacteria,
  - Innate Immunity to Intracellular Bacteria,
  - Adaptive Immunity to Intracellular Bacteria,
  - Immune Evasion by Intracellular Bacteria,
- Immunity to Fungi,
  - Innate Immunity to Fungi,
  - Adaptive Immunity to Fungi,
- Immunity to Viruses,
  - Innate Immunity to Viruses,
  - Adaptive Immunity to Viruses,

  - Immune Evasion by Viruses,

- Immunity to Parasites,
  - Innate Immunity to Parasites,
  - Adaptive Immunity to Parasites,
  - Immune Evasion by Parasites,

- Strategies for Vaccine Development,
  - Attenuated and Inactivated Bacterial and Viral Vaccines,
  - Purified Antigen (Subunit) Vaccines,
  - Synthetic Antigen Vaccines,
  - Live Viral Vaccines Involving Recombinant Viruses,
  - DNA Vaccines,
  - mRNA Vaccines,
  - Adjuvants and Immunomodulators,
  - Passive Immunization,

- Summary,

In the preceding chapters, we have referred to protection against infections as the major physiologic function of the immune system and discussed immune responses in the context of responses to microbes. In this chapter, we will integrate this information and discuss the main features of immunity to different types of pathogenic microorganisms, as well as the mechanisms microbes use to resist immune defenses.

The development of an infectious disease in an individual involves complex interactions between the microbe and the host. The key events during infection include entry of the microbe, invasion and colonization of host tissues, evasion of host immunity, and tissue injury or functional impairment. Microbes produce disease by killing the host cells they infect or releasing toxins that can cause tissue damage and functional derangements in neighboring or distant cells and tissues that are not infected. In addition, microbes often cause disease by stimulating innate and adaptive immune responses that kill the microbes, but also may injure and impair the function of normal tissues. Many features of microorganisms determine their virulence, and many diverse mechanisms contribute to the pathogenesis of infectious diseases. The topic of microbial pathogenesis is beyond the scope of this book. Our discussion will focus on host immune responses to pathogenic microorganisms.

Overview of Immune Responses to Microbes

Microbes can cause disease of varying severity and chronicity (Fig. 16.1). In many instances, an infection is asymptomatic and resolves without intervention. A typical host immune response is rapidly triggered by the infection, wanes as the infection is cleared, and leaves memory cells that can provide long-term protection. Recurrent illnesses occur when the host response cannot eradicate the microbe and may even contribute to the disease. Although antimicrobial host defense reactions are numerous and varied, there are several important general features of immunity to microbes.

Defense against microbes is mediated by the effector mechanisms of innate and adaptive immunity. The innate immune system provides early defense, and the adaptive immune system provides a more sustained and stronger response as well as protection against repeat infection by the same microbe. Many pathogenic microbes have evolved to resist innate immunity, and protection against such infections is critically dependent on adaptive immune responses. Adaptive responses are effective against even virulent microbes because they generate large numbers of effector cells and antibody molecules that function to eliminate the microbes and memory cells that protect the individual from repeated infections. However, it typically takes several days for adaptive immune responses to develop fully (the time needed for naive lymphocyte proliferation and differentiation into effector cells), and innate immunity provides critical defense during this window.

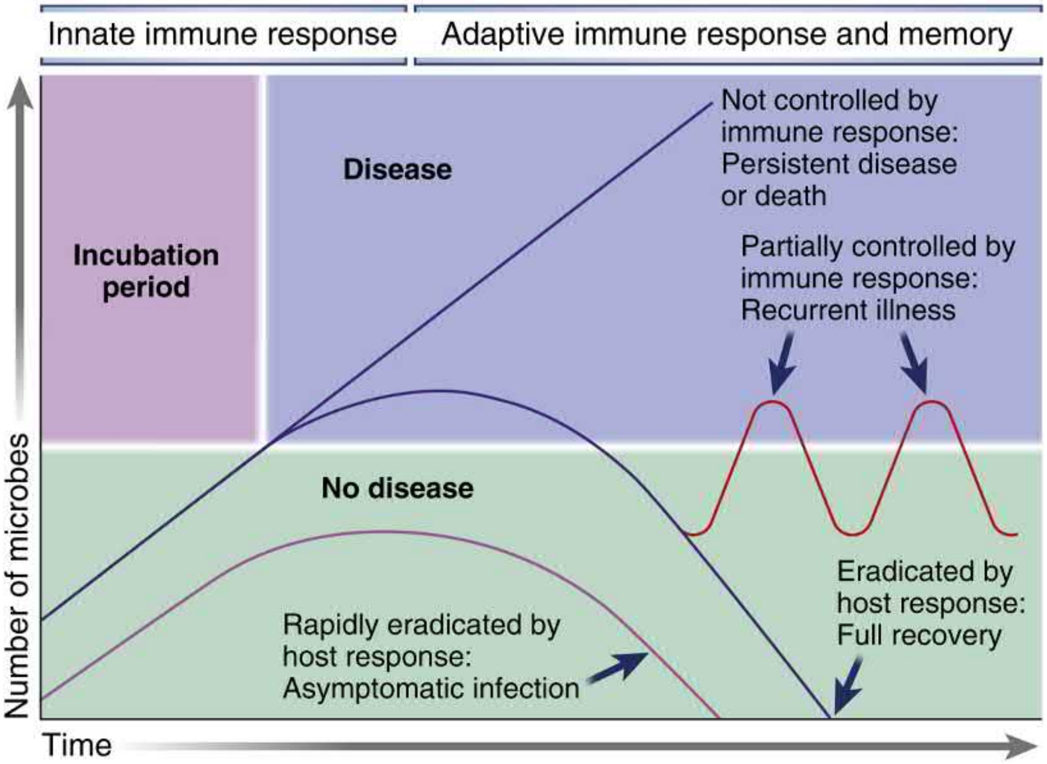


FIGURE 16.1 The progression and outcome of infections. Most infections have an incubation period followed by disease that may be short-lived if the host response is effective or progressive or chronic if the response does not adequately control the infection.

Modified from Engleberg NC, DiRita V, Dermody TS, eds. *Schaechter's Mechanisms of Microbial Disease.* 5th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2013.

The immune system responds in specialized and distinct ways to different types of microbes to combat these infectious agents most effectively. Different microbes require different mechanisms for elimination, and the adaptive immune system has evolved to respond in the optimal way to a vast diversity of microbes. The generation of different subsets of effector CD4$^+$ T cells (see Chapter 10) and the production of different isotypes of antibodies (see Chapter 12) are excellent examples of the specialization of adaptive immunity.

The survival and pathogenicity of microbes in a host are critically influenced by the ability of the microbes to evade or resist the effector mechanisms of immunity. As we will see later in this chapter, microorganisms have developed a variety of mechanisms for surviving in the face of powerful immunologic defenses. Infectious microbes and the immune system have co-evolved and are engaged in a constant struggle for survival. The balance between host immune responses and microbial strategies for resisting immunity often determines the outcome of infections.

Some microbes establish latent, or persistent, infections in which the immune response controls but does not eliminate the microbe. Several viruses, especially DNA viruses of the herpesvirus and poxvirus families, and some intracellular bacteria are capable of establishing latent infections. In latent viral infections, the viral DNA persists in the genome of infected cells but no infectious virus is produced. In persistent bacterial infections, such as tuberculosis, the bacteria may survive within the phagocytic vesicles of infected macrophages. In all of these situations, some latent microbes will on occasion become activated and start replicating, especially if the immune system is weakened for any reason.

In many infections, tissue injury and disease may be caused by the host response to the microbe rather than by the microbe itself. Immunity is necessary for host survival but also has the potential for causing injury to the host.

Inherited and acquired defects in innate and adaptive immunity are important causes of susceptibility to infections. Common acquired causes of immunodeficiency include human immunodeficiency virus (HIV) infection and therapeutic immunosuppression by drugs to treat inflammatory and autoimmune diseases or prevent transplant rejection. Although less common, there are a large number of inherited immunodeficiency syndromes caused by mutations in single genes whose major clinical consequence is increased infections. In addition, subtle and poorly defined defects in host defenses may underlie common infections. We will describe immunodeficiencies in detail in Chapter 21. Aging individuals also show increased susceptibility to infection, presumably because of reduced adaptive immune responses. Paradoxically, with age there may be increased inflammation without overt triggers.

Infections in the population elicit immune responses that establish herd immunity, which protects against the spread of the infection. Conversely, newly emerging infections are able to spread in part because of the absence of herd immunity, resulting in epidemics and pandemics. Most of these new infections are caused by viruses that develop novel strains because of reassortment between the genomes of existing human strains and animal strains. (This process is discussed in more detail later, when we consider how influenza viruses escape from immune defense.) The most devastating pandemic in recent history was caused by the influenza virus in 1918 to 1920, estimated to have killed 20 to 50 million people in Western Europe and other regions of the world. More recently, widespread infections have been caused by the coronavirus that caused severe acute respiratory distress syndrome (SARS) in 2002; a new influenza strain in 2009; the coronavirus that caused Middle Eastern respiratory syndrome (MERS), which emerged in 2012; the Zika virus in 2015 (which causes neurologic defects in newborns); and the coronavirus SARS-CoV-2 from 2019 onward (which causes the disease called COVID-19). The rapid spread of the COVID-19 pandemic, which has caused over 2 million deaths by early 2021 and major disruptions in societies worldwide, demonstrates the extreme danger of emerging infections in populations that lack preexisting immunity.

Analysis of immune responses is a valuable clinical assay for infections. The most useful test is measurement of serum antibodies specific for particular microbes. This is helpful for detecting infections in which the microbe cannot be cultured, is not detectable in the blood by molecular assays, or is present in tissues that are not readily accessible. The presence of IgM (immunoglobulin M) antibodies is indicative of recent infection, whereas the presence of only IgG suggests past infection, reflecting the sequence of antibody isotype production (IgM followed by IgG) in response to any immunization (see Chapter 12). Other tests include assays for T cell responses, such as tests for skin reactions to microbial antigens and cytokine (e.g., interferon-γ [IFN-γ]) release after activation of peripheral blood cells with antigens, used to detect infection with *Mycobacterium tuberculosis*.

TABLE 16.1

Microbe	Examples of Human Diseases	Mechanisms of Pathogenicity
Extracellular Bacteria		
Staphylococcus aureus	Skin and soft-tissue infections, lung abscess Systemic: toxic shock syndrome Food poisoning	Skin infections: acute inflammation induced by toxins; cell death caused by pore-forming toxins Systemic: toxin (“superantigen”)-induced cytokine production by T cells causing skin necrosis, shock, diarrhea
***Streptococcus pyogenes* (group A)**	Pharyngitis Skin infections: impetigo, erysipelas, cellulitis Systemic: scarlet fever	Acute inflammation induced by various toxins (e.g., streptolysin O damages cell membranes)
***Streptococcus pneumoniae* (pneumococcus)**	Pneumonia, meningitis	Acute inflammation induced by cell wall constituents; pneumolysin is similar to streptolysin O
Escherichia coli	Urinary tract infections, gastroenteritis, septic shock	Toxins induce intestinal epithelial chloride and water secretion; endotoxin (LPS) stimulates cytokine secretion by macrophages
Vibrio cholerae	Diarrhea (cholera)	Cholera toxin ADP-ribosylates G protein subunit, leading to increased cAMP in intestinal epithelial cells resulting in chloride secretion and water loss
Clostridium tetani	Tetanus	Tetanus toxin binds to the motor end plate at neuromuscular junctions and causes irreversible muscle contraction
Corynebacterium diphtheriae	Diphtheria	Diphtheria toxin ADP-ribosylates elongation factor 2 and inhibits protein synthesis
Facultative Intracellular Bacteria		
Mycobacterium tuberculosis	Tuberculosis	Macrophage activation resulting in granulomatous inflammation and tissue destruction
Salmonella typhi	Typhoid	Enterocolitis

Table Continued

Microbe	**Examples of Human Diseases**	**Mechanisms of Pathogenicity**
Neisseria meningitidis **(meningococcus)**	Meningitis	Acute inflammation and systemic disease caused by potent toxin
Listeria monocytogenes	Listeriosis	Listeriolysin damages cell membranes
Legionella pneumophila	Legionnaires' disease	Cytotoxin lyses cells and causes lung injury and inflammation
Obligate Intracellular Bacteria		
Mycobacterium leprae	Leprosy	Destructive or granulomatous lesions associated with varying degrees of cell-mediated immune responses
Chlamydia	Urogenital and eye infections	Acute inflammation
Rickettsia	Typhus, other diseases	Endothelial infection and dysfunction
Extracellular Fungi		
Candida albicans	Candidiasis	Acute inflammation; binds complement proteins
Aspergillus fumigatus	Aspergillosis	Invasion and thrombosis of blood vessels causing ischemic necrosis and cell injury
Intracellular Fungi		
Histoplasma capsulatum	Histoplasmosis	Lung infection causes granulomatous inflammation
Pneumocystis jiroveci	Pneumonia	Impaired macrophage clearance in setting of impaired T cell immunity, leading to alveolar inflammation
Cryptococcus neoformans	Cryptococcosis	Multiple virulence factors
Viruses		
Polio	Poliomyelitis	Inhibits host cell protein synthesis (tropism for motor neurons in the anterior horn of the spinal cord)
Influenza	Pneumonia	Inhibits host cell protein synthesis (tropism for ciliated epithelium)
Rabies	Encephalitis	Inhibits host cell protein synthesis (tropism for peripheral nerves)
Herpes simplex	Various herpes infections (skin, systemic)	Inhibits host cell protein synthesis; functional impairment of immune cells
Hepatitis (A, B, C)	Viral hepatitis	Host CTL response to infected hepatocytes
Epstein-Barr virus	Infectious mononucleosis; B cell proliferation, lymphomas	Acute infection: cell lysis (tropism for B lymphocytes) Latent infection: stimulates B cell proliferation
HIV	AIDS	Multiple: killing of CD4$^+$ T cells, functional impairment of immune cells (see Chapter 20)

ADP, Adenosine diphosphate; *AIDS*, acquired immunodeficiency syndrome; *cAMP,* cyclic adenosine monophosphate; *CTL,* cytotoxic T lymphocyte; *HIV*, human immunodeficiency virus; *LPS,* lipopolysaccharide.

This table was compiled with the assistance of Dr. Arlene Sharpe, Department of Pathology, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts.

[a] Examples of pathogenic microbes of different classes are listed, with brief summaries of known or postulated mechanisms of tissue injury and disease. Facultative intracellular bacteria can live inside or outside cells, whereas obligate intracellular organisms can live and replicate only inside cells. Examples of parasites are listed in Table 16.4.

In this chapter, we will consider the main features of immunity to five categories of pathogenic microorganisms: extracellular bacteria, intracellular bacteria, fungi, viruses, and protozoan and multicellular parasites (Table 16.1; see also Table 16.4 later in the chapter). This separation provides a useful context for discussing immunity because the dominant immune responses to these types of infections are different. We use the terms extracellular and intracellular bacteria to refer to where the organisms survive and replicate; even extracellular bacteria become intracellular when they are ingested into phagocytes, but they do not survive inside phagocytes. Our discussion of the immune responses to these microbes illustrates the physiologic significance of the effector functions of lymphocytes discussed in earlier chapters.

Immunity to Extracellular Bacteria

Extracellular bacteria are capable of replicating outside host cells, for example, in the blood, in connective tissues, and in tissue spaces such as the lumens of the airways and gastrointestinal tract. Many different species of extracellular bacteria are pathogenic, and disease is caused by two principal mechanisms. First, these bacteria induce inflammation, which results in tissue injury at the site of infection. Second, bacteria produce toxins, which have diverse pathologic effects. The toxins include an endotoxin, which is the lipopolysaccharide (LPS) complex in the cell wall of gram-negative bacteria, and exotoxins, which are secreted by many types of bacteria. Endotoxin activates macrophages and other cells and stimulates the production of cytokines that mediate host defense and also can cause disease. Many exotoxins are cytotoxic and kill host cells, and others cause disease by various mechanisms. For instance, diphtheria toxin shuts down protein synthesis in infected cells, cholera toxin interferes with ion and water transport, tetanus toxin inhibits neuromuscular transmission, and anthrax toxin disrupts several critical biochemical signaling pathways in infected cells.

Innate Immunity to Extracellular Bacteria

The principal mechanisms of innate immunity to extracellular bacteria are phagocytosis, complement activation, and the inflammatory response.

- ***Activation of phagocytes and inflammation.*** Extracellular bacteria are efficiently killed by phagocytes (neutrophils and macrophages) because these microbes have not adapted to surviving inside these cells. Therefore, recruitment and activation of phagocytes to the site of infection, which is part of the inflammatory response, is the major defense mechanism against these microbes. Phagocytes are recruited and activated in response to bacterial products, which act directly on the phagocytes and also induce secretion of cytokines that act on the cells. Tissue-resident dendritic cells (DCs) and macrophages that are activated by the microbes secrete cytokines that promote leukocyte infiltration into sites of infection. Recruited neutrophils and blood monocyte–derived macrophages, as well as tissue-resident macrophages, use surface receptors, including mannose receptors and scavenger receptors, to recognize extracellular bacteria, and they use Fc receptors and complement receptors to recognize bacteria opsonized with antibodies and complement proteins, respectively. Microbial products activate Toll-like receptors (TLRs) and other pattern recognition receptors in phagocytes and other cells. Some of these receptors function mainly to promote the phagocytosis of the microbes (e.g., mannose receptors, scavenger receptors); others stimulate the microbicidal activities of the phagocytes (mainly TLRs); and yet others promote both phagocytosis and activation of the phagocytes (Fc and complement receptors) (see Chapter 4). The activated phagocytes ingest microbes and destroy them mainly in phagolysosomes.
- ***Complement activation.*** Bacteria provide a surface for complement activation by the alternative pathway (see Chapters 4 and 13). Bacteria express mannose on their surface that binds to mannose-binding lectin, or N-acetyl compounds that bind to ficolins, both of which activate complement by the lectin pathway (see Chapter 4). One

result of complement activation is opsonization and enhanced phagocytosis of the bacteria. In addition, the membrane attack complex (MAC) generated by complement activation lyses bacteria, especially *Neisseria* species, which are particularly susceptible to lysis because of their thin cell walls, and complement by-products stimulate inflammatory responses by recruiting and activating leukocytes.

- Innate lymphoid cells (ILCs), γδ T cells, and NKT cells have all been described in infections, mostly in experimental models, but their role in host defense, especially in humans, is not established.

Adaptive Immunity to Extracellular Bacteria

Humoral immunity is a major protective immune response against extracellular bacteria, and it functions to block infection, to eliminate the microbes, and to neutralize their toxins (Fig. 16.2A) **.** Antibody responses against extracellular bacteria are directed against cell wall antigens and toxins, which may be polysaccharides or proteins. The polysaccharides are T-independent antigens that elicit antibody responses but do not activate T cells. Therefore, humoral immunity is the principal mechanism of defense against polysaccharide-rich encapsulated bacteria. For these microbes, including *Streptococcus pneumoniae, Neisseria* species, and others, the spleen plays a major role in both production of the antibodies and phagocytic clearance of the opsonized bacteria. People whose spleens are either surgically removed because of trauma or other reasons or are damaged in hematologic disorders are at increased risk for severe infections by these encapsulated bacteria. Protein antigens, which are present in or secreted by most bacteria, elicit more potent isotype-switched, high-affinity antibodies, as well as cell-mediated immunity. The effector mechanisms used by antibodies to combat infections include neutralization, opsonization and phagocytosis, and activation of complement by the classical pathway (see Chapter 13). Neutralization is mediated by high-affinity IgG, IgM, and IgA isotypes, the latter mainly in the lumens of mucosal organs. Opsonization is mediated by the IgG1 and IgG3 subclasses of IgG, and complement activation is initiated by IgM, IgG1, and IgG3.

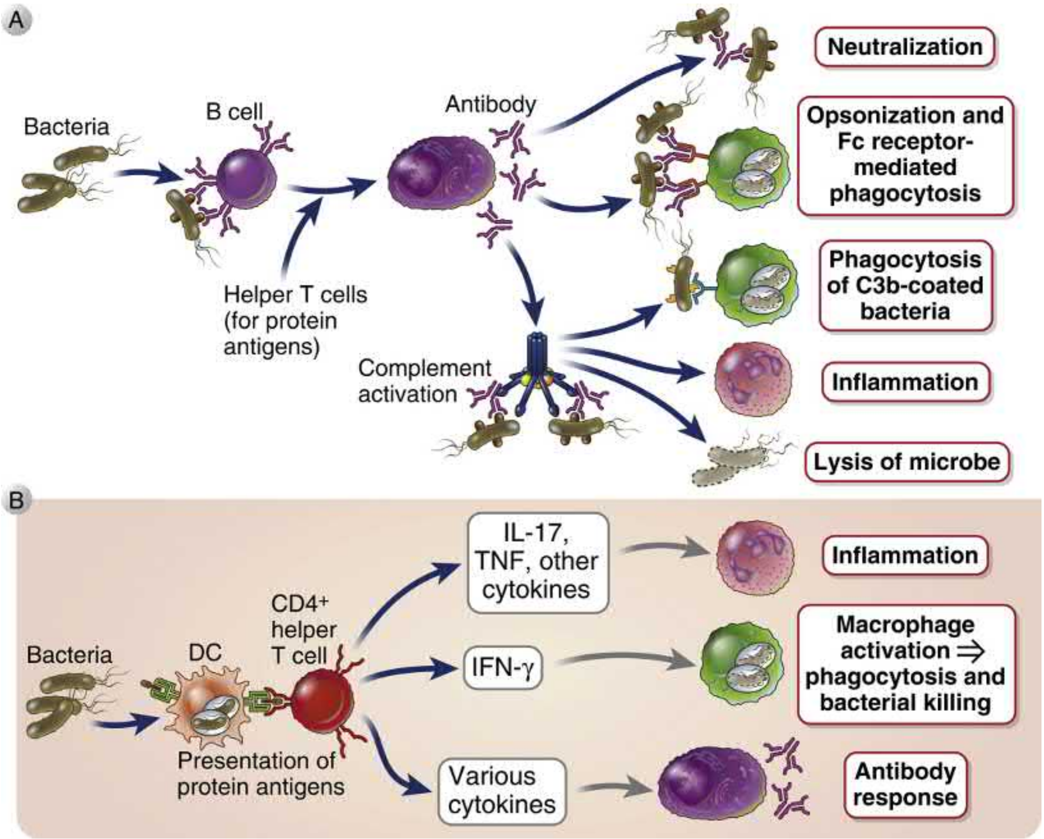

A
Bacteria
B cell
Antibody
Helper T cells (for protein antigens)
Complement activation
Neutralization
Opsonization and Fc receptor-mediated phagocytosis
Phagocytosis of C3b-coated bacteria
Inflammation
Lysis of microbe
B
Bacteria
DC
CD4+ helper T cell
Presentation of protein antigens
IL-17, TNF, other cytokines
IFN-γ
Various cytokines
Inflammation
Macrophage activation ⇒ phagocytosis and bacterial killing
Antibody response

FIGURE 16.2 Adaptive immune responses to extracellular microbes.Adaptive immune responses to extracellular microbes such as bacteria and their toxins consist of antibody production **(A)** and the activation of CD4$^+$ helper T cells, which work by secreted cytokines **(B)** and CD40-ligand (not shown). Antibodies neutralize and eliminate microbes and toxins by several mechanisms. Helper T cells produce cytokines that stimulate inflammation, macrophage activation, and B cell responses. *DC*, Dendritic cell; *IFN-γ,* interferon-gamma; *IL,* interleukin; *TNF,* tumor necrosis factor.

The protein antigens of extracellular bacteria also activate CD4$^+$ helper T cells, which produce cytokines and express cell surface molecules that induce local inflammation, enhance the phagocytic and microbicidal activities of macrophages and neutrophils, and

stimulate antibody production (Fig. 16.2B) **.** Th17 responses induced by these microbes recruit neutrophils and monocytes and thus promote local inflammation at sites of bacterial infection. Patients with genetic defects in Th17 development and those who make neutralizing autoantibodies specific for IL-17 have increased susceptibility to extracellular bacterial and fungal infections and develop multiple skin abscesses.

Injurious Effects of Immune Responses to Extracellular Bacteria

The principal injurious consequences of host responses to extracellular bacteria are inflammation and sepsis. The same reactions of neutrophils and macrophages that function to eradicate the infection also cause tissue damage by local production of reactive oxygen species (ROS) and lysosomal enzymes. These inflammatory reactions are usually self-limited and controlled. Cytokines secreted by leukocytes in response to bacterial products also stimulate the production of acute-phase proteins and cause the systemic manifestations of the infection (see Chapter 4). **Sepsis** is a pathologic consequence of severe, local or disseminated infection by some gram-negative and gram-positive bacteria, as well as some fungi. Sepsis typically manifests clinically with abnormalities in tissue blood perfusion, coagulation, metabolism, and organ function. Septic shock is the most severe and frequently fatal form of sepsis, characterized by circulatory collapse (shock) and disseminated intravascular coagulation. The early phase of bacterial sepsis is caused by cytokines produced by macrophages that are activated by bacterial cell wall components, including LPS and peptidoglycans. Tumor necrosis factor (TNF), IL-6, and IL-1 are the principal cytokine mediators of sepsis, but IFN-γ and IL-12 may also contribute (see Chapter 4). This early burst of large amounts of cytokines is sometimes called a cytokine storm. There is some evidence that in LPS-induced sepsis, activation of a noncanonical inflammasome pathway causes cell death and release of inflammatory mediators (the process called pyroptosis, see Chapter 4), and this is essential for development of the disease.

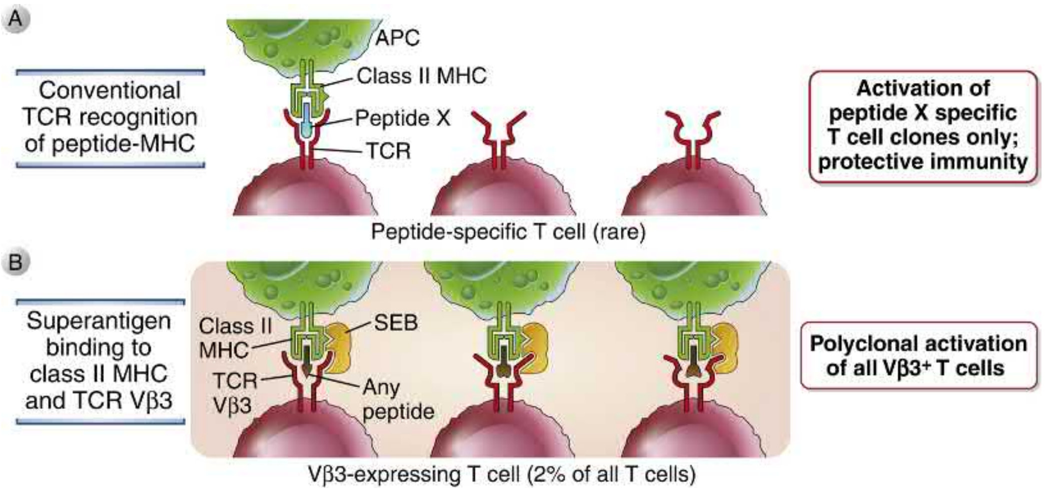

A
Conventional TCR recognition of peptide-MHC
APC
Class II MHC
Peptide X
TCR
Activation of peptide X specific T cell clones only; protective immunity
Peptide-specific T cell (rare)
B
Superantigen binding to class II MHC and TCR Vβ3
Class II MHC
SEB
TCR Vβ3
Any peptide
Polyclonal activation of all Vβ3⁺ T cells
Vβ3-expressing T cell (2% of all T cells)

FIGURE 16.3 Polyclonal activation of T cells by bacterial superantigens. **A,** Conventional microbial T cell antigens, composed of a peptide bound to the peptide-binding groove of a major histocompatibility complex *(MHC)* molecule, are recognized by a very small fraction of T cells in any one individual, and only these T cells are activated to become effector T cells that protect against the microbe. **B,** In contrast, a superantigen binds to class II MHC molecules outside the peptide-binding groove, in an allele-nonspecfic manner, and simultaneously binds to the variable region of many different T cell receptor *(TCR)* β chains, regardless of the peptide specificity of the TCR. Different superantigens bind to TCRs of different Vβ families. Because many T cells express a TCR β chain from a particular Vβ family, superantigens can activate a large number of T cells. In the example shown, the superantigen staphylococcal enterotoxin B *(SEB)* binds to human leukocyte antigen (HLA)-DR and the V regions of TCRs belonging to the Vβ3 family. Other superantigens may bind different class II MHC molecules and

TCRs of different Vβ families. *APC,* Antigen-presenting cell.

Certain bacterial toxins stimulate all T cells that express members of a particular T cell receptor (TCR) Vβ gene family. Such toxins are called **superantigens** because, like the typical antigens T cells recognize, they bind to TCRs and to class II major histocompatibility complex (MHC) molecules (although not to the peptide-binding clefts), but they activate many more clones of T cells than do conventional peptide antigens (Fig. 16.3). Their importance lies in their ability to activate many T cells, with the subsequent production of large amounts of cytokines that can also cause a sepsis-like systemic inflammatory response syndrome.

A late complication of the humoral immune response to bacterial infection may be the generation of disease-producing antibodies. The best-defined examples are two rare sequelae of streptococcal infections of the throat or skin that are manifested weeks or even months after the infections are controlled. Rheumatic fever is a sequel to pharyngeal infection with group A β-hemolytic streptococci. Infection leads to the production of antibodies and activation of T cells specific for bacterial cell wall proteins. Some of these antibodies and T cells cross-react with myocardial proteins and cause inflammation affecting the heart muscle (myocarditis) and the valves (endocarditis). Poststreptococcal glomerulonephritis is a sequel to infection of the skin or throat with nephritogenic strains of group A β-hemolytic streptococci. Antibodies produced against these bacteria form complexes with bacterial antigen, which may be deposited in kidney glomeruli and cause nephritis.

Immune Evasion by Extracellular Bacteria

The virulence of extracellular bacteria has been linked to a number of mechanisms that enable the microbes to resist innate immunity (Table 16.2). Bacteria with polysaccharide-rich capsules resist phagocytosis and are therefore more virulent than homologous strains lacking a capsule. The capsules of many pathogenic gram-positive and gram-negative bacteria contain sialic acid residues that inhibit complement activation by the alternative pathway.

A mechanism used by bacteria to evade humoral immunity is variation of surface antigens (Fig. 16.4) **.** Some surface antigens of bacteria, such as gonococci and *Escherichia coli,* are contained in their pili, which are the structures responsible for bacterial adhesion to host cells. The major antigen of the pili is a protein called pilin. The pilin genes of gonococci undergo extensive gene conversion, because of which the progeny of one organism can produce up to 10^6 antigenically distinct pilin molecules. This ability to alter antigens helps the bacteria to evade attack by pilin-specific antibodies, although its principal significance for the bacteria may be to select for pili that are more adherent to host cells so that the bacteria are more virulent. Changes in the production of glycosidases lead to chemical alterations in surface oligosaccharides, which enable the bacteria to evade humoral immune responses against these antigens. Bacteria also release surface antigens in membrane blebs, which may divert antibodies away from the microbes themselves.

TABLE 16.2

Mechanism of Immune Evasion	Examples
Extracellular Bacteria	
Antigenic variation	*Neisseria gonorrhoeae, Escherichia coli, Salmonella typhimurium*
Inhibition of complement activation	Many bacteria
Resistance to phagocytosis	Pneumococcus, *Neisseria meningitidis*
Scavenging of reactive oxygen species	Catalase-positive bacteria (including staphylococci and many others)
Intracellular Bacteria	
Inhibition of phagolysosome formation	*Mycobacterium tuberculosis, Legionella pneumophila*
Inactivation of reactive oxygen and nitrogen species	*Mycobacterium leprae* (phenolic glycolipid)
Disruption of phagosome membrane, escape into cytoplasm	*Listeria monocytogenes* (hemolysin protein)

Immunity to Intracellular Bacteria

Intracellular bacteria are those that are ingested by macrophages but are able to survive and even replicate within these cells. Because these microbes find a niche where they are inaccessible to circulating antibodies, their elimination requires the mechanisms of cell-mediated immunity (Fig. 16.5). As we will discuss later in this section, in many intracellular bacterial infections the host response also causes tissue injury. Most bacteria do not infect nonphagocytic cells as do viruses. Therefore, a primary mechanism of host defense against such bacteria is to activate phagocytes to destroy the ingested microbes. Some bacteria do infect nonphagocytic cells, including *Rickettsia* species that infect endothelial cells and *Chlamydia* species that infect epithelial cells. A mechanism of defense against these bacteria is killing of infected cells by cytotoxic T lymphocytes (CTLs).

Innate Immunity to Intracellular Bacteria

The innate immune response to intracellular bacteria is mediated mainly by phagocytes and NK cells. Phagocytes, initially neutrophils and later macrophages, ingest and attempt to destroy these microbes, but pathogenic intracellular bacteria are resistant to degradation within phagocytes. Products of these bacteria are recognized by TLRs and cytoplasmic proteins of the NOD-like receptor (NLR) family, resulting in activation of the phagocytes (see Chapter 4). Bacterial DNA and cyclic dinucleotides produced by the bacteria in the cytosol stimulate type I IFN responses through the STING pathway.

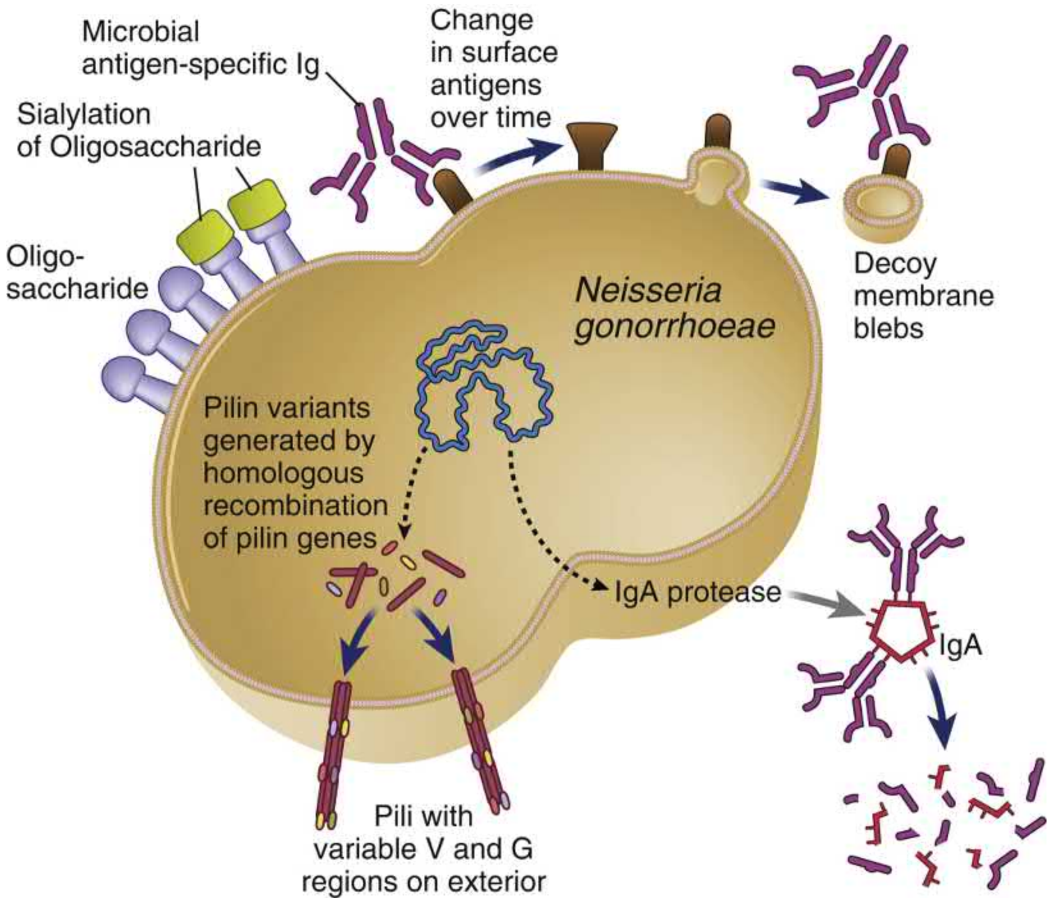

Microbial antigen-specific Ig
Change in surface antigens over time
Sialylation of Oligosaccharide
Oligo-saccharide
Neisseria gonorrhoeae
Decoy membrane blebs
Pilin variants generated by homologous recombination of pilin genes
IgA protease
IgA
Pili with variable V and G regions on exterior

FIGURE 16.4 Mechanisms of immune evasion in bacteria.Shown are the multiple mechanisms used by one bacterial species, *Neisseria,* to evade humoral immunity. *Ig,* Immunoglobulin.

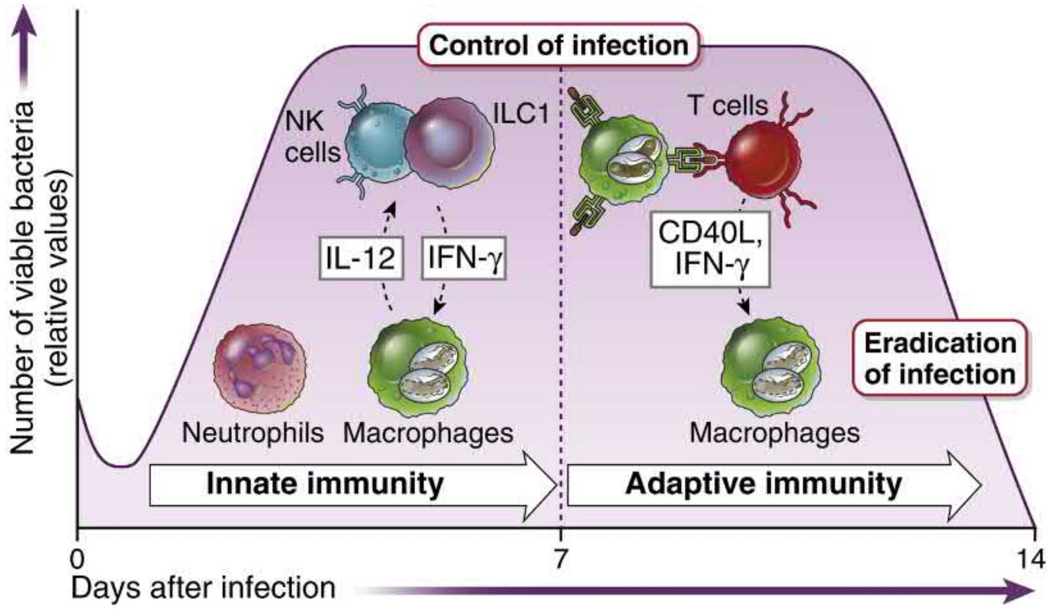

Number of viable bacteria (relative values)
Control of infection
NK cells
ILC1
IL-12
IFN-γ
Neutrophils
Macrophages
T cells
CD40L, IFN-γ
Macrophages
Eradication of infection
Innate immunity
Adaptive immunity
0
7
14
Days after infection

FIGURE 16.5 Innate and adaptive immunity to intracellular bacteria.The innate immune response to intracellular bacteria consists of phagocytes and natural killer *(NK)* cells, interactions among which are mediated by cytokines (interleukin-12 *[IL-12]* and interferon-γ *[IFN-γ]*). The typical adaptive immune response to these microbes is cell-mediated immunity, in which T cells activate phagocytes to eliminate the microbes. Innate immunity may control bacterial growth, but elimination of the bacteria requires adaptive immunity. These principles are based largely on analysis of *Listeria monocytogenes* infection in mice; the numbers of viable bacteria shown on the *y*-axis are relative values of bacterial colonies that can be grown from the tissues of infected mice.

Intracellular bacteria activate NK cells by inducing expression of NK cell–activating ligands on infected cells and by stimulating DC and macrophage production of IL-12 and IL-15, both of which are NK cell–activating cytokines. The NK cells produce IFN-γ, which in turn activates macrophages and promotes killing of the phagocytosed bacteria. Thus, NK cells provide an early defense against these microbes, before the development of adaptive immunity. In fact, mice with severe combined immunodeficiency, which lack T and B cells, are able to transiently control infection with the intracellular bacterium *Listeria monocytogenes* by NK cell–derived IFN-γ production. However, innate immunity usually fails to eradicate these infections, and eradication requires adaptive cell-mediated immunity.

The ability of type 1 ILCs to defend against intracellular bacteria has been demonstrated in mouse models. These ILCs secrete IFN-γ and TNF, which activate macrophages and help to clear intracellular pathogens.

Adaptive Immunity to Intracellular Bacteria

The major protective immune response against intracellular bacteria is T cell–mediated recruitment and activation of phagocytes (cell-mediated immunity). Individuals with deficient cell-mediated immunity, such as patients with AIDS (acquired immunodeficiency syndrome), are extremely susceptible to infections with intracellular bacteria (as well as intracellular fungi and viruses). Many of the important features of cell-mediated immunity were established in the 1950s based on studies of immune responses to the intracellular bacterium *L. monocytogenes* in mice. This form of immunity could be adoptively transferred to naive animals with lymphocytes but not with serum from infected or immunized animals (see Fig. 10.3).

As we discussed in Chapters 10 and 11, T cells provide defense against infections by two types of reactions: CD4$^+$ Th1 cells activate phagocytes through the actions of CD40 ligand and IFN-γ, resulting in killing of microbes that are ingested by phagocytes into phagolysosomes, and CD8+ CTLs kill infected cells, eliminating microbes that escape the killing mechanisms of phagocytes. CD4$^+$ T cells differentiate into Th1 effectors under the influence of IL-12, which is produced by macrophages and DCs. The T cells express CD40 ligand and secrete IFN-γ, and these two stimuli activate macrophages to produce several microbicidal substances, including nitric oxide, lysosomal enzymes, and ROS. These substances are produced mainly within phagolysosomes and thus target microbes ingested into the vesicles. The importance of IL-12 and IFN-γ in immunity to intracellular bacteria has been demonstrated in experimental models and in congenital immunodeficiencies. For instance, individuals with inherited mutations in receptors for IFN-γ or IL-12 are highly susceptible to infections with normally avirulent mycobacteria (see Chapter 21).

Numerous cytokines in addition to IFN-γ play important roles in defense against intracellular bacteria such as *M. tuberculosis*. TNF, produced by activated macrophages and other cells, recruits and activates mononuclear phagocytes to combat mycobacteria; this is why patients with rheumatoid arthritis and other autoimmune diseases who are treated with TNF antagonists become susceptible to mycobacterial infections.

In some infections, the bacteria escape from vesicles and enter the cytoplasm of infected cells. For instance, *Listeria* produces a protein that creates holes in the phagosomal membrane and enables the bacteria to escape into the cytosol, thus evading the microbicidal mechanisms of the phagocytes (which are concentrated in the phagolysosomes). For eradication of such infections, the infected cells have to be killed by CTLs, which recognize cytosolic peptides displayed by class I MHC molecules. Thus, the effectors of cell-mediated immunity, namely CD4$^+$ T cells that activate macrophages and CD8$^+$ CTLs, function cooperatively in defense against intracellular bacteria (Fig. 16.6).

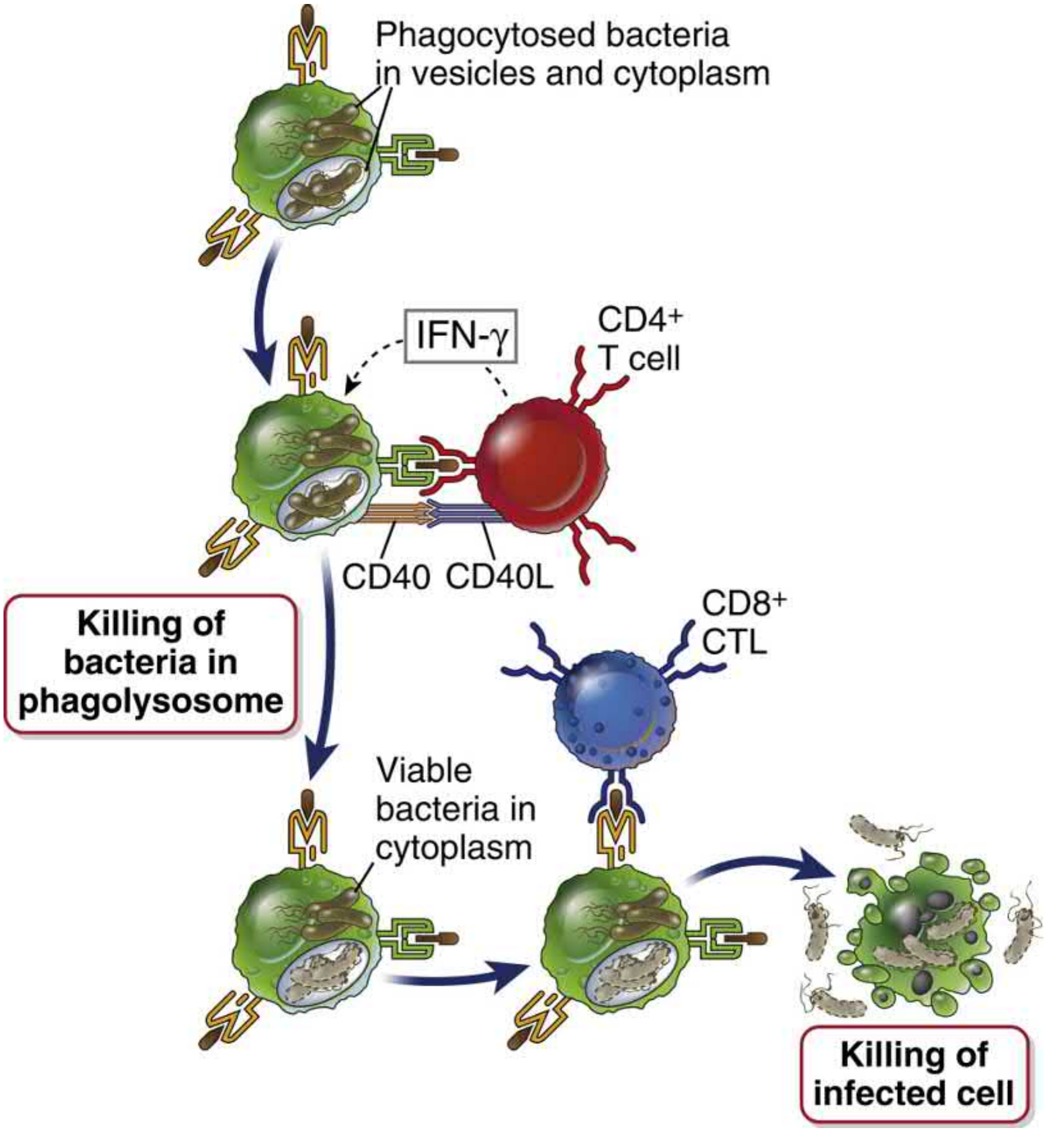

Phagocytosed bacteria in vesicles and cytoplasm
IFN-γ
CD4+ T cell
CD40
CD40L
Killing of bacteria in phagolysosome
CD8+ CTL
Viable bacteria in cytoplasm
Killing of infected cell

FIGURE 16.6 Cooperation of CD4$^+$ and CD8$^+$ T cells in defense against intracellular microbes. Intracellular bacteria such as *L. monocytogenes* are phagocytosed by macrophages and may survive in phagosomes and escape into the cytoplasm. CD4$^+$ T cells respond to class II major histocompatibility complex (MHC)-associated peptide antigens derived from the intravesicular bacteria. These T cells produce interferon-γ *(IFN-γ)* and express CD40 ligand, which activates macrophages to destroy the microbes in phagosomes. CD8$^+$ T

cells respond to class I–associated peptides derived from cytosolic antigens and kill the infected cells. *CTL*, Cytologic T cell.

The macrophage activation that occurs in response to intracellular microbes is capable of causing tissue injury. This injury may be the result of delayed-type hypersensitivity (DTH) reactions to microbial protein antigens (see Chapter 19). Because intracellular bacteria have evolved to resist killing within phagocytes, they often persist for long periods and cause chronic T cell and macrophage activation, which may result in the formation of granulomas surrounding the microbes (see Fig. 19.8). The histologic hallmark of infection with some intracellular bacteria is granulomatous inflammation. This type of inflammatory reaction may serve to localize and prevent spread of the microbes, but it is also associated with severe functional impairment caused by tissue necrosis and fibrosis. In fact, necrotizing granulomas and the fibrosis (scarring) that accompanies granulomatous inflammation are important causes of tissue injury and clinical disease in tuberculosis. Individuals who have been previously infected with *M. tuberculosis* show cutaneous DTH reactions to skin challenge with a bacterial antigen preparation (purified protein derivative [PPD]). This is the basis of a commonly used skin test to detect previous infection.

Differences among individuals in the patterns of T cell responses to intracellular microbes are important determinants of disease progression and clinical outcome. Leprosy, which is caused by *Mycobacterium leprae,* is considered an example of the relationship between the type of T cell response and disease outcome in humans. There are two polar forms of leprosy, the lepromatous and tuberculoid forms, although many patients fall into less clear intermediate groups. In lepromatous leprosy, patients have high specific antibody titers but weak cell-mediated responses to *M. leprae* antigens. Mycobacteria proliferate within macrophages and are detectable in large numbers. The bacterial growth and persistent but inadequate macrophage activation result in destructive lesions in the skin and underlying tissue. In contrast, patients with tuberculoid leprosy have strong cell-mediated immunity but low antibody levels. This pattern of immunity is reflected in granulomas that form around nerves and produce peripheral sensory nerve defects and secondary traumatic skin lesions but with less tissue destruction and a paucity of bacteria in the lesions. One possible reason for the differences in these two forms of disease caused by the same organism may be that there are different patterns of T cell differentiation and cytokine production in individuals. Some studies indicate that patients with the tuberculoid form of the disease produce IFN-γ and IL-2 in lesions (indicative of Th1 cell activation), whereas patients with lepromatous leprosy produce less IFN-γ and may exhibit weak cell-mediated immunity and failure to control bacterial spread. The role of Th1- and Th2-derived cytokines in determining the outcome of infection has been most clearly demonstrated in infection by the protozoan parasite *Leishmania major* in different strains of inbred mice (discussed later in this chapter).

Immune Evasion by Intracellular Bacteria

Intracellular bacteria have developed various strategies to resist elimination by phagocytes (see Table 16.2). These include inhibiting phagolysosome fusion or escaping into the cytosol, and directly scavenging or inactivating microbicidal substances, such as ROS. The outcome of infection by these organisms often depends on whether the T cell–stimulated antimicrobial mechanisms of macrophages or microbial resistance to killing gain the upper hand. Resistance to phagocyte-mediated elimination is also the reason that such bacteria tend to cause chronic infections that are difficult to eradicate. Some of these infections, notably tuberculosis, may last for years. The bacteria survive in a quiescent latent state within phagocytes and often become active, especially if the immune system is compromised.

Immunity to Fungi

Fungal infections, also called mycoses, are important causes of morbidity and mortality in humans. Some fungal infections are endemic, and these infections are usually caused by fungi that are present in the environment and whose spores enter humans. Other fungal infections are said to be opportunistic because the etiologic agents cause mild or no disease in healthy individuals but may infect and cause severe disease in immunodeficient persons. Compromised immunity is the most important predisposing factor for fungal infections that cause serious illness. Neutrophil deficiency as a result of bone marrow suppression or damage is frequently associated with such infections. Opportunistic fungal infections are also associated with immunodeficiency caused by HIV and by therapy for disseminated cancer and transplant rejection.

Different fungi infect humans and may live outside of cells in many tissues or within phagocytes. Therefore, the immune responses to these microbes are often combinations of the responses to extracellular and intracellular microbes.

Innate Immunity to Fungi

The principal mediators of innate immunity against fungi are phagocytes and the complement system (Fig. 16.7) **.** Phagocytosis by neutrophils and macrophages is important for controlling fungal infections in tissues. These phagocytes detect fungal molecules by a variety of pattern recognition receptors, including TLRs and lectins such as Dectin and mannose receptors (see Chapter 4). Neutrophils seem to be especially important in defense against predominantly extracellular fungi, and disseminated fungal infections are frequent complications of conditions associated with neutropenia, such as leukemias and cancer therapies that compromise bone marrow function. Neutrophils and macrophages liberate fungicidal substances, such as ROS and lysosomal enzymes, and phagocytose fungi for intracellular killing.

Complement activation is mainly involved in defense against fungi that enter the bloodstream, such as *Candida* organisms. Fungi may activate the alternative or lectin complement pathways. Complement products opsonize fungi for phagocytosis but may not directly lyse fungi because of their thick cell walls, which resist killing by the MAC.

Adaptive Immunity to Fungi

Th17 cells play a critical role in adaptive immunity to extracellular fungal infections in tissues (see Fig. 16-7) **.** Fungi such as *Candida* frequently infect epithelial tissues such as the skin and the oropharyngeal mucosa; other fungi often infect the gastrointestinal tract. In all of these locations, fungi induce Th17 responses, in large part because fungal carbohydrates stimulate DCs and macrophages by receptors such as Dectin to liberate cytokines that promote Th17 differentiation (see Chapter 10). Th17 cells recruit neutrophils that phagocytose and destroy the fungi. Predictably, the most prominent complication of inherited mutations affecting Th17 development is chronic mucocutaneous candidiasis.

Th1-mediated cellular immunity is an important defense mechanism against intracellular fungal infections. *Histoplasma capsulatum,* a facultative intracellular parasite that lives in macrophages, is eliminated by the same cellular mechanisms that are effective against intracellular bacteria. $CD4^+$ Th1 and $CD8^+$ T cells cooperate to eliminate the yeast forms of *Cryptococcus neoformans,* which tend to colonize the lungs and brain in immunodeficient hosts. *Pneumocystis jiroveci* is another intracellular fungus that causes serious infections in individuals with defective cell–mediated immunity, such as patients with AIDS; bone marrow failure, often resulting from leukemias; and inherited deficiency in CD40-ligand (see Chapter 21).

Th2 responses are thought to be ineffective at clearing fungi and may cause harm. In *Aspergillus* infection of the airways, Th2 responses against the fungus cause a serious inflammatory reaction called allergic bronchopulmonary aspergillosis.

Little is known about how fungi evade host immunity. Virulent strains of *C. neoformans* inhibit the production of cytokines, such as

TNF and IL-12 by macrophages, and stimulate production of IL-10, thus inhibiting macrophage activation.

Immunity to Viruses

Viruses are obligatory intracellular microorganisms that use components of the nucleic acid and protein synthetic machinery of the host to replicate. Viruses typically infect various cell types by receptor-mediated endocytosis after binding to normal cell surface molecules. Viruses can cause tissue injury and disease by any of several mechanisms. Viral replication interferes with normal cellular protein synthesis and function and leads to injury and ultimately death of the infected cell. This result is one type of cytopathic effect of viruses, and the infection is said to be lytic because the infected cell is lysed. Viruses can stimulate inflammatory responses that cause damage to tissues. Viruses may also cause latent infections, discussed later.

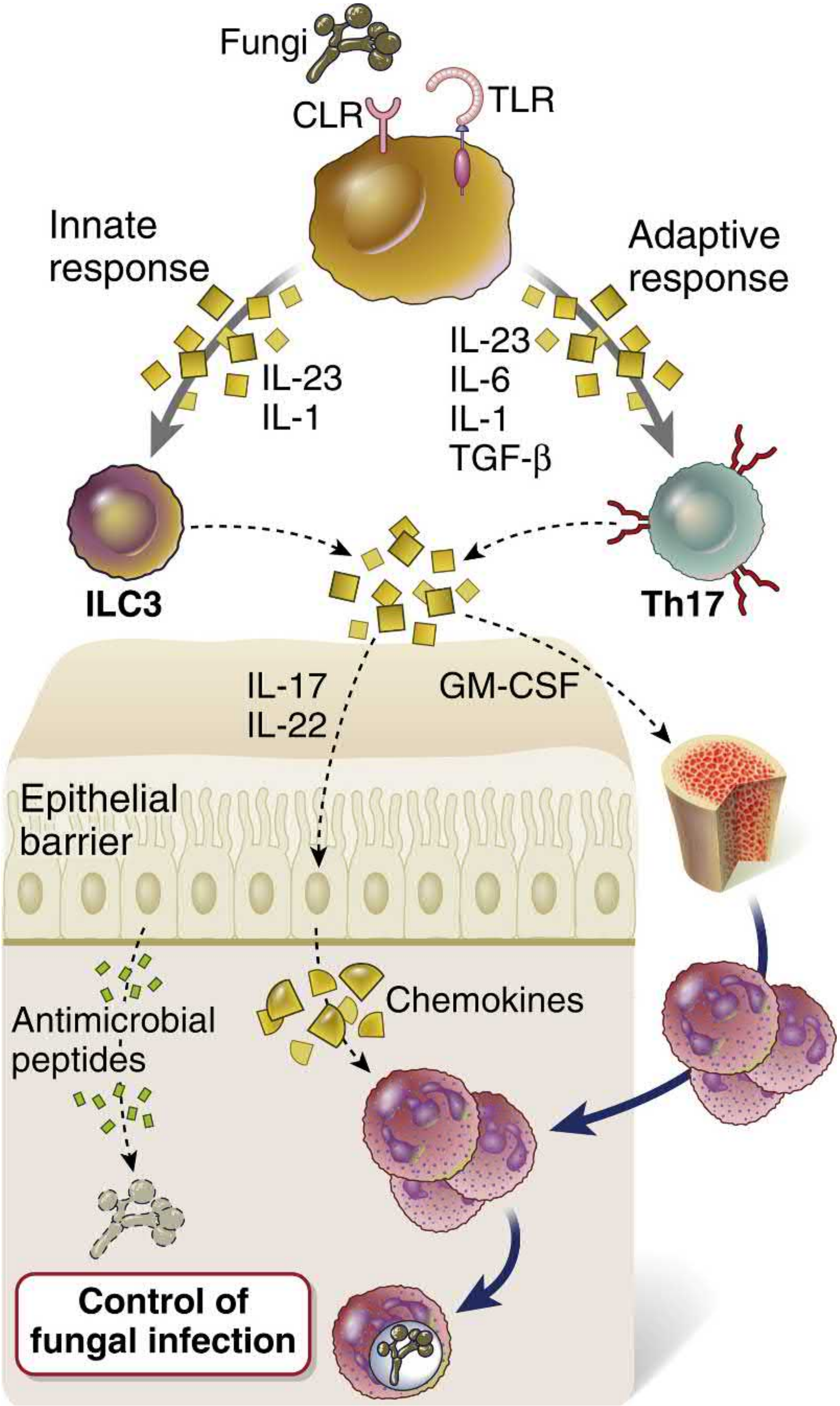

Fungi
CLR
TLR
Innate response
Adaptive response
IL-23
IL-1
IL-23
IL-6
IL-1
TGF-β
ILC3
Th17
IL-17
IL-22
GM-CSF
Epithelial barrier
Antimicrobial peptides
Chemokines
Control of fungal infection

FIGURE 16.7 Role of innate immunity and Th17 cells in defense against fungal infection. Dendritic cells (DCs) and macrophages (not shown) recognize fungal glucans and liberate cytokines that stimulate innate lymphoid cells *(ILC3s)* resident in the tissues to release cytokines, mainly interleukin-17 *(IL-17),* which recruit neutrophils and induce the production of antimicrobial peptides that protect against the infection. Cytokines may directly recruit neutrophils as well. The DCs also stimulate the differentiation of naive fungal antigen-specific $CD4^+$ T cells into Th17 cells in draining lymph nodes, and the Th17 cells migrate back to the site of infection. Granulocyte-macrophage colony-stimulating factor *(GM-CSF)* produced by the ILCs (and perhaps Th17 cells) may contribute to recruitment of neutrophils. *CLR,* C-type lectin receptor (e.g., dectin-1); *TGF-β,* transforming growth factor-β; *TLR,* Toll-like receptor.

Innate and adaptive immune responses to viruses are aimed at blocking infection and eliminating infected cells (Fig. 16.8).

Innate Immunity to Viruses

The principal mechanisms of innate immunity against viruses are inhibition of infection by type I IFNs and NK cell–mediated killing of infected cells. Infection by many viruses is associated with production of type I IFNs by infected cells and by plasmacytoid DCs and macrophages responding to viral products (see Chapter 4). Several biochemical pathways trigger IFN production. These include recognition of viral RNA and DNA by endosomal TLRs and activation of cytoplasmic RIG-like receptors and the STING pathway by viral RNA and DNA, respectively (Fig. 16.9). These pathways converge on the activation of protein kinases, which in turn activate the IRF transcription factors that stimulate IFN gene transcription. Type I IFNs inhibit viral replication in both infected and uninfected cells. The mechanisms by which these cytokines block viral replication were discussed in Chapter 4 (see Fig. 4.18). Type I IFNs also stimulate the production of other host proteins that interfere with viral replication and are called restriction factors. The role of restriction factors in defense against viruses is best understood for HIV (see Chapter 21).

NK cells kill virus-infected cells and are an important mechanism of immunity against viruses, especially DNA viruses (herpesviruses, human papilloma virus [HPV], and others) early in the course of infection before adaptive immune responses have developed. Class I MHC expression is often shut off in virus-infected cells as an escape mechanism from CTLs. This enables NK cells to kill the infected cells because the absence of class I releases NK cells from a normal state of inhibition (see Fig. 4.10). Viral infection may also stimulate expression of activating NK cell ligands on the infected cells.

Adaptive Immunity to Viruses

Adaptive immunity against viral infections is mediated by antibodies that block virus binding and entry into host cells and by CTLs, which eliminate the infection by killing infected cells (see Fig. 16.8) **.** The most effective antibodies are high-affinity antibodies produced in T-dependent germinal center reactions (see Chapter 12). Antibodies are effective against viruses only during the extracellular stage of the lives of these microbes. Viruses will be extracellular when they first enter the body before they infect host cells, or when they are released from infected cells by virus budding or if the infected cells die. Antiviral antibodies bind to viral envelope or capsid antigens and function mainly as neutralizing antibodies to prevent virus attachment and entry into host cells. Thus, antibodies prevent both initial infection and cell-to-cell spread. Secreted antibodies, especially of the IgA isotype, are important for neutralizing viruses within the respiratory and intestinal tracts. Oral immunization against poliovirus works by inducing mucosal immunity. In addition to neutralization, antibodies may opsonize viral particles and promote their clearance by phagocytes. Complement activation may also participate in antibody-mediated viral immunity, mainly by promoting phagocytosis and possibly by direct lysis of viruses with lipid envelopes.

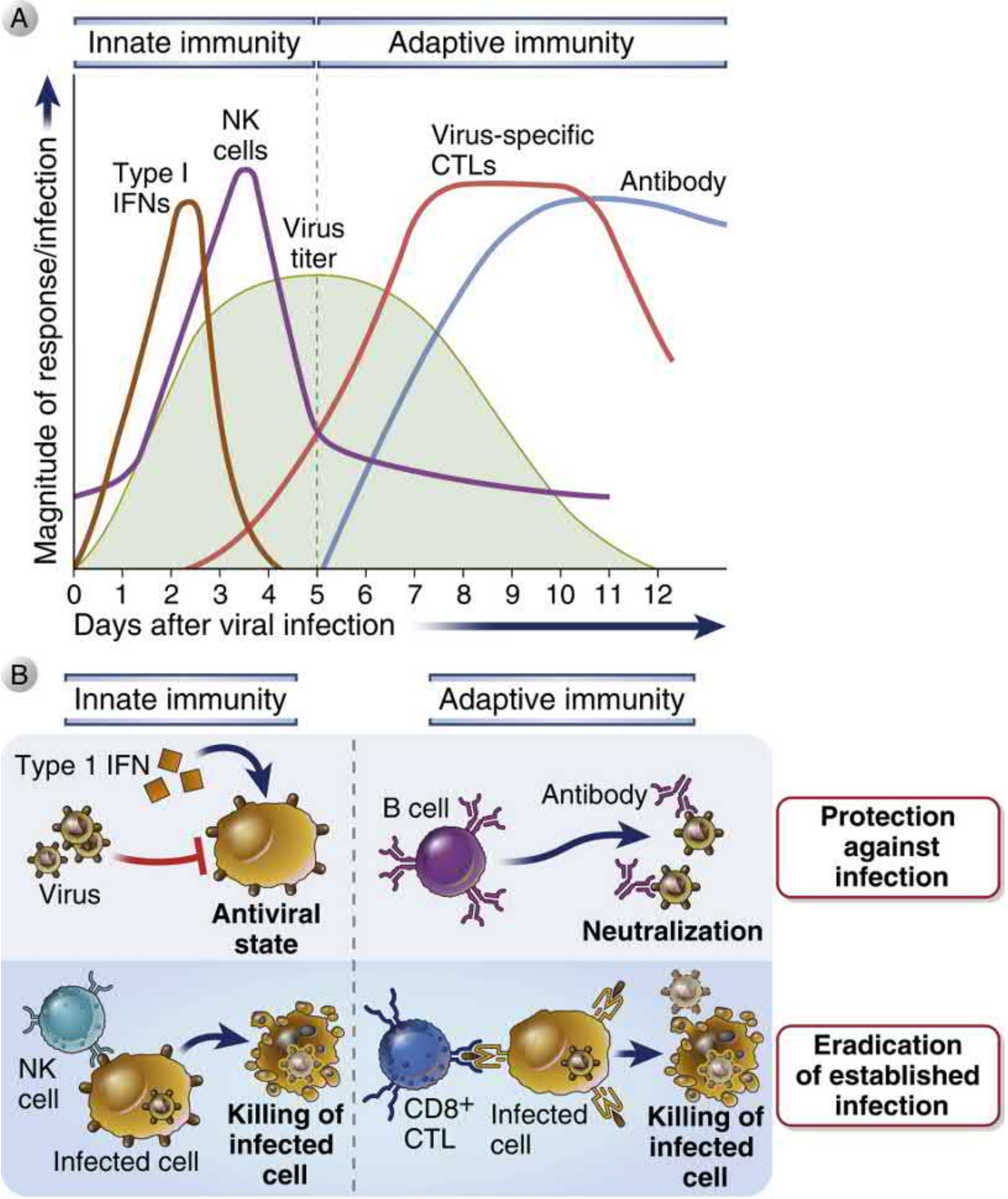

A
Innate immunity
Adaptive immunity
Magnitude of response/infection
Type I IFNs
NK cells
Virus titer
Virus-specific CTLs
Antibody
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after viral infection
B
Innate immunity
Adaptive immunity
Type 1 IFN
Virus
Antiviral state
B cell
Antibody
Neutralization
Protection against infection
NK cell
Infected cell
Killing of infected cell
CD8+ CTL
Infected cell
Killing of infected cell
Eradication of established infection

FIGURE 16.8 Innate and adaptive immune responses against viruses. **A,** Kinetics of innate and adaptive immune responses to a virus infection. **B,** Mechanisms by which innate and adaptive immunity prevent and eradicate virus infections. Innate immunity is mediated by type 1 interferons *(IFNs),* which prevent infection, and natural killer *(NK)* cells, which eliminate infected cells. Adaptive immunity is mediated by antibodies

and cytotoxic T lymphocytes *(CTLs)*, which block infection and kill infected cells, respectively.

The importance of humoral immunity in defense against viral infections is supported by the observation that resistance to a particular virus, induced by either infection or vaccination, is often specific for the serologic (antibody-defined) type of the virus. An example is influenza virus, in which exposure to one serologic type does not confer resistance to other serotypes of the virus. Neutralizing antibodies block viral infection of cells and spread of viruses from cell to cell, but after the viruses enter cells and begin to replicate intracellularly, they are inaccessible to antibodies. Therefore, humoral immunity induced by previous infection or vaccination is able to protect individuals from viral infection but cannot by itself eradicate established infection.

Elimination of viruses that reside within cells is mediated by CTLs, which kill the infected cells. As we have mentioned in previous chapters, the principal physiologic function of CTLs is surveillance against viral infection. Most virus-specific CTLs are $CD8^+$ T cells that recognize cytosolic, usually endogenously synthesized, viral peptides presented by class I MHC molecules. If the infected cell is a tissue cell and not an antigen-presenting cell (APC), such as a DC, the infected cell or viral proteins released from the cell may be phagocytosed by the DC, which processes the viral antigens and presents them to naive $CD8^+$ T cells to initiate the antiviral T cell response. We described this process of cross-presentation, or cross-priming, in Chapter 6 (see Fig. 6.14). Full differentiation of $CD8^+$ CTLs often requires cytokines produced by $CD4^+$ helper cells or costimulators expressed on APCs (see Chapter 11). As discussed in Chapters 9 and 11, $CD8^+$ T cells undergo massive proliferation during viral infection, and most of the proliferating cells are specific for only a few viral peptides. The activated T cells differentiate into effector CTLs, which can kill any infected nucleated cell that is producing viral antigens in the cytosol and presenting peptides from these antigens on cell surface class I MHC molecules. The antiviral effects of CTLs are mainly due to killing of infected cells, but other mechanisms include activation of nucleases within infected cells that degrade viral genomes and secretion of cytokines, such as IFN-γ, which activates phagocytes.

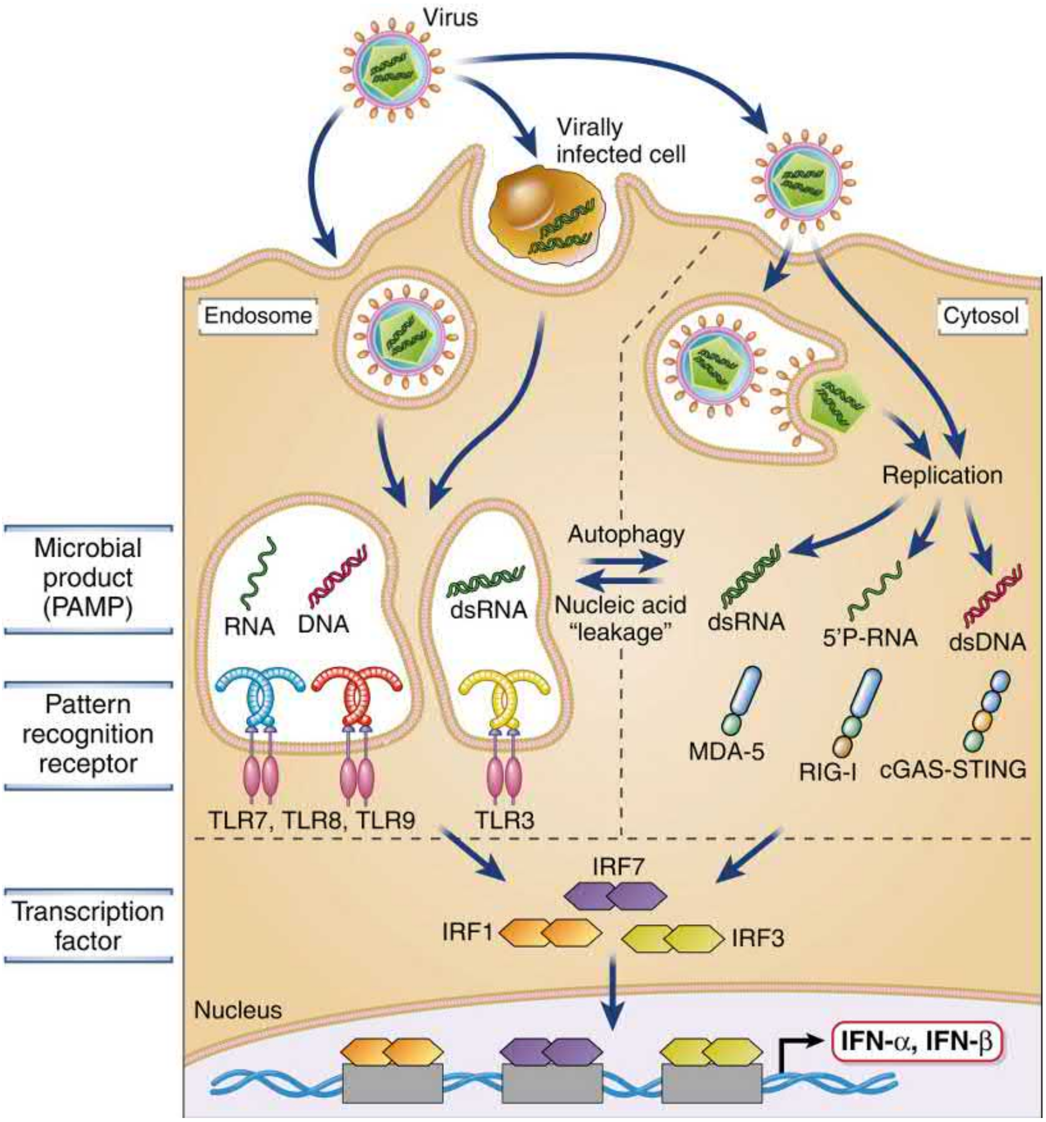

Virus
Virally infected cell
Endosome
Cytosol
Replication
Microbial product (PAMP)
RNA
DNA
dsRNA
Autophagy
Nucleic acid "leakage"
dsRNA
5'P-RNA
dsDNA
Pattern recognition receptor
MDA-5
RIG-I
cGAS-STING
TLR7, TLR8, TLR9
TLR3
IRF7
Transcription factor
IRF1
IRF3
Nucleus
IFN-α, IFN-β

FIGURE 16.9 Production of type I interferons. Viral nucleic acids in infected cells activate numerous pathways that lead to the production of antiviral type I interferons *(IFNs).* If viruses enter the cell by endocytosis, viral RNA and DNA can bind to Toll-like receptors *(TLRs)* in the endosomal membrane, which induces signals that activate IFN response factors *(IRFs)* and type I IFN gene expression. During viral replication, viral RNA in the cytosol can activate retinoic acid–inducible gene *(RIG)*-like receptors, such as melanoma differentiation-associated gene 5 *(MDA5)* and

RIG-1, and viral dsDNA can activate the cGAS-STING pathway, both leading to IRF activation and type I IFN gene expression. *ds,* Double stranded; *cGAS,* cyclic guanosine monophosphate–adenosine monophosphate; *STING,* stimulator of IFN genes.

Many lines of experimental and clinical evidence support the importance of CTLs in defense against viral infection. Susceptibility to such infections is increased in patients and animals deficient in T lymphocytes. Humans and experimental animals can be protected against some virus infections by adoptive transfer of virus-specific, class I–restricted CTLs. Viruses have developed numerous strategies to escape attack by $CD8^+$ CTLs. These include blocking the processing and presentation of antigens by the class I MHC pathway and shutting down $CD8^+$ T cell responses by inducing the phenomenon of exhaustion. These evasion mechanisms are discussed later in the chapter.

In latent infections, viral DNA persists in host cells, but the virus does not replicate or kill infected cells. Latency is a feature of infections by several viruses, especially DNA viruses of the herpesvirus and poxvirus families. In latent viral infections, the viral DNA may be integrated into the DNA of infected cells, but no infectious virus is produced. The host immune response controls but does not eliminate the infection. If the host's immune system becomes defective for any reason, the virus may be reactivated, causing significant clinical disease. Examples include shingles due to reactivation of latent varicella infection and cold sores due to reactivation of latent herpes simplex virus.

In some viral infections, tissue injury may be caused by CTLs. Some degree of immunopathology accompanies host responses to many, perhaps most, virus infections. An experimental model of a disease in which the pathologic condition is primarily due to the host immune response is lymphocytic choriomeningitis virus (LCMV) infection in mice, which induces inflammation of the spinal cord meninges. LCMV infects meningeal cells, but it is noncytopathic and does not injure the infected cells directly. The virus stimulates the development of virus-specific CTLs that kill infected meningeal cells during a physiologic attempt to eradicate the infection. Therefore, meningitis develops in normal mice with intact immune systems, but T cell–deficient mice do not develop disease and instead become carriers of the virus. This observation appears to contradict the usual situation, in which immunodeficient individuals are more susceptible to infectious diseases than are normal individuals. Hepatitis B virus infection in humans has the unusual feature that immunodeficient persons who become infected often do not develop the disease but become carriers who can transmit the infection to otherwise healthy persons. The livers of patients with acute and chronic active hepatitis contain large numbers of CD8$^+$ T cells, and hepatitis virus–specific, class I MHC–restricted CTLs can be isolated from liver biopsy specimens and propagated in vitro. These findings support the view that the CTL response is the main cause of tissue injury in viral hepatitis.

SARS-CoV-2 sometimes induces a systemic inflammatory reaction that contributes to the pathology and clinical manifestations of COVID-19. The inflammation is associated with increased production of numerous cytokines. In some patients, the inflammation is severe and life-threatening. Severe disease is characterized by largely myeloid cell–driven inflammation, with exuberant monocyte activation and cytokine production induced by viral RNA and possibly products of dead cells. Often, there is severe lymphopenia with decreased T cell numbers. The contribution of adaptive immune cells to inflammation in this disease is not well established, nor is it known why this particular coronavirus has a propensity to elicit such a reaction. It is also not clear why severe disease is seen in older individuals, especially in men with preexisting conditions such as type 2 diabetes.

Immune responses to viral infections may be involved in producing disease in other ways. Antiviral antibodies may promote activation of macrophages and other myeloid cells through binding to Fc receptors and even enhance the entry of viruses into host cells. This reaction, called antibody-mediated enhancement, has been postulated to increase lung inflammation associated with some virus infections, such as coronaviruses, dengue, and respiratory syncytial virus. A consequence of persistent infection with some viruses, such as hepatitis B, is the formation of circulating immune complexes composed of viral antigens and specific antibodies (see Chapter 19). These complexes are deposited in blood vessels and lead to systemic vasculitis. Some coronaviruses that cause severe life-threatening disease may induce endothelial damage and activate coagulation, thus inducing local thrombus formation and tissue damage. Some viral proteins contain amino acid sequences that are also present in some self antigens. It has been postulated that because of this molecular mimicry, antiviral immunity can lead to immune responses against self antigens.

Immune Evasion by Viruses

Viruses have evolved numerous mechanisms for evading host immunity (Table 16.3).

- ***Viruses can alter their antigens and are thus no longer targets of immune responses.*** The antigens affected are most commonly surface glycoproteins that are recognized by antibodies, but T cell epitopes may also undergo variation. The principal mechanisms of antigenic variation are point mutations and reassortment of RNA genomes (in RNA viruses), leading to antigenic drift and antigenic shift. These processes are of great importance in the spread of influenza virus. The two major antigens of the virus are the trimeric viral hemagglutinin (the viral spike protein) and neuraminidase. Viral genomes undergo mutations in the genes that encode these surface proteins, and the variation that occurs as a result is called **antigenic drift.** Alternatively, the segmented RNA genomes of various strains of influenza viruses that normally inhabit different host species can recombine in host cells, and these reassorted viruses can differ quite dramatically from prevalent strains (Fig. 16.10). Reassortment of viral genes results in major changes in antigenic structure called **antigenic shift,** which creates distinct viruses such as the avian flu or the swine flu viruses. Because of antigenic variation, a virus may become resistant to immunity generated in the population by previous infections. The influenza pandemics that occurred in 1918, 1957, and 1968 were due to different strains of the virus, and the H1N1 pandemic of 2009 was due to a strain in which the strands of the RNA genome were reassorted among strains endemic in pigs, fowl, and humans. Subtler viral variants arise more frequently. There are so many serotypes of rhinovirus that vaccination against the common cold may not be a feasible preventive strategy. HIV-1, which causes AIDS, is also capable of tremendous antigenic variation because of a high error rate in reverse transcription of its RNA genome during viral reproduction (see Chapter 21). In these situations, prophylactic vaccination may have to be directed against invariant viral proteins.

TABLE 16.3

Mechanism of Immune Evasion	Examples
Antigenic variation	Influenza, rhinovirus, HIV
Inhibition of antigen processing **Blockade of TAP transporter** **Removal of class I molecules from the ER**	HSV CMV
Production of "decoy" MHC molecules to inhibit NK cells	Cytomegalovirus (murine)
Production of cytokine receptor homologs	Vaccinia, poxviruses (IL-1, IFN-γ), cytomegalovirus (chemokine)
Production of immunosuppressive cytokine	Epstein-Barr (IL-10)
Infection and death or functional impairment of immune cells	HIV
Inhibition of complement activation **Recruitment of factor H** **Incorporation of CD59 in viral envelope**	HIV HIV, vaccinia, human CMV
Inhibition of innate immunity **Inhibition of access to RIG-I RNA sensor** **Inhibition of PKR (signaling by IFN receptor)**	Vaccinia, HIV HIV, HCV, HSV, polio

Representative examples of different mechanisms used by viruses to resist host immunity are listed.

CMV, Cytomegalovirus; *ER,* endoplasmic reticulum; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HSV,* Herpes simplex virus; *IFN,* interferon; *IL,* interleukin; *MHC,* major histocompatibility complex; *NK cells,* natural killer cells; *TAP,* transporter associated with antigen processing.

- ***Some viruses inhibit the ability of the host to induce the antiviral state.*** The coronaviruses SARS-CoV, MERS-CoV, and SARS-CoV-2 can all shut off the host type I IFN response. Among the proteins produced by SARS-CoV-2, 10 of 29 are dedicated to dampening or preventing host type I IFN production. They do so by various means. Some viral proteins modify viral RNA to make it more closely resemble host mRNA (by adding a version of a 7-methyl guanosine cap and by adding a 2′O-methy group to the ribose in the next nucleotide). This prevents recognition by RIG-I and MDA-5 (see Chapter 4). Other proteins attenuate the translation of host type I IFNs.

The importance of type I IFN as an antiviral mechanism is further highlighted by the observation that individuals who make autoantibodies against their own type I IFN or have genetic defects affecting IFN production or signaling are at increased risk for developing severe COVID-19 following infection with SARS-CoV-2.

- ***Some viruses inhibit class I MHC – associated presentation of cytosolic protein antigens.*** Viruses make a variety of proteins that block different steps in antigen processing, transport, and presentation (Fig. 16.11). Inhibition of antigen presentation blocks the assembly and expression of stable class I MHC molecules and the display of viral peptides. As a result, cells infected by such viruses cannot be recognized or killed by $CD8^+$ CTLs. As discussed earlier, NK cells are activated by infected cells, especially in the absence of class I MHC molecules. Some viruses may produce proteins that act as ligands for NK cell inhibitory receptors and thus inhibit NK cell activation.

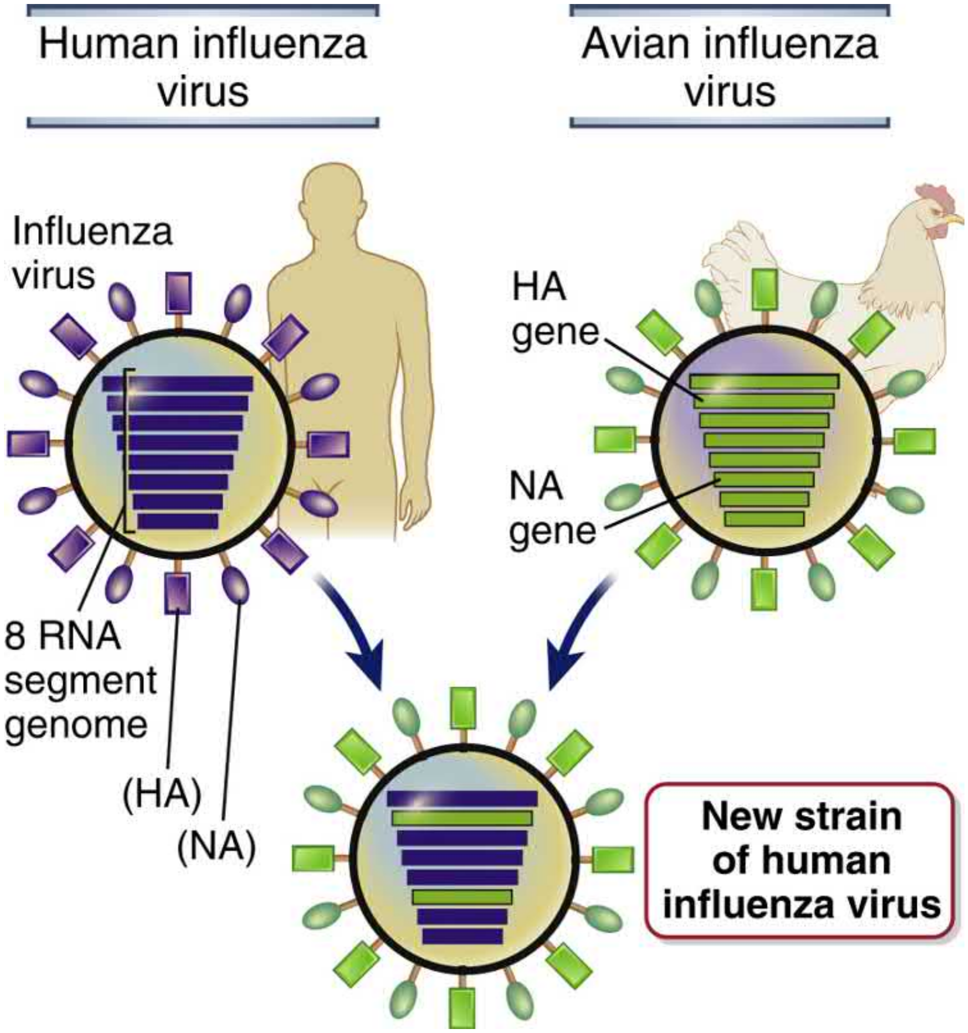

Human influenza virus
Avian influenza virus
Influenza virus
HA gene
NA gene
8 RNA segment genome
(HA)
(NA)
New strain of human influenza virus

FIGURE 16.10 Generation of new influenza virus strains by genetic recombination (antigenic shift).The genome of the influenza virus is composed of eight separate RNA strands, which allows genetic recombination by reassortment of the segments in various hosts, such as pigs (not shown), birds, or humans, that are simultaneously infected with two different strains. These genetic reassortments create new viruses that are antigenically distinct from their precursors and thus are able to evade immune detection in large numbers of newly infected hosts. The H1N1 influenza virus, which was responsible

for the pandemic of 2009, was generated by reassortment of swine, avian, and human viruses in pigs and then passed back to humans. *HA,* Hemagglutinin; *NA,* neuraminidase.

- ***Some viruses produce molecules that inhibit the effector phase of immune responses.*** Poxviruses encode molecules that are secreted by infected cells and bind to several cytokines, including IFN-γ, TNF, IL-1, IL-18, and chemokines. The secreted cytokine-binding proteins may function as competitive antagonists of the cytokines. Epstein-Barr virus produces a protein that is homologous to the cytokine IL-10, which inhibits activation of macrophages and DCs and may thus suppress cell-mediated immunity. These examples probably represent a small fraction of immunosuppressive viral molecules. Identification of these molecules raises the intriguing possibility that viruses have acquired genes encoding endogenous inhibitors of immune responses during their passage through human hosts and have thus evolved to infect and colonize humans.
- ***Some chronic viral infections are associated with failure of CTL responses, called exhaustion, which allows viral persistence.*** Studies of a chronic infection with LCMV in mice have shown that this type of immune deficit may result from persistent antigen stimulation leading to upregulation of T cell inhibitory receptors, such as PD-1 (programmed cell death protein 1) (see Fig. 11.3). There is evidence for $CD8^+$ T cell exhaustion in chronic human viral infections, including HIV and hepatitis virus infection. The physiologic importance of PD-1–mediated T cell inhibition may be that it limits pathology caused by strong immune responses to viruses.
- ***Viruses may infect and either kill or inactivate immunocompetent cells.*** The obvious example is HIV, which

survives by infecting and eliminating $CD4^+$ T cells, the key inducers of immune responses to protein antigens.

Immunity to Parasites

Parasites include single-celled protozoa, complex multicellular worms (helminths), and ectoparasites (e.g., ticks and mites). Parasitic infections are major health problems, particularly in lower-income countries. It is estimated that approximately 30% of the world's population suffers from parasitic infestations. There are approximately 200 million new malaria cases each year worldwide and approximately 400,000 deaths annually. The magnitude of this public health problem is the principal reason for the great interest in immunity to parasites and for the development of immunoparasitology as a distinct branch of immunology.

Most parasites go through complex life cycles, part of which occurs in humans (or other vertebrates) and part of which occurs in intermediate hosts, such as flies, ticks, and snails. Humans are usually infected by bites from infected intermediate hosts or by sharing a particular habitat with an intermediate host. For instance, malaria and trypanosomiasis are transmitted by insect bites, and schistosomiasis is transmitted by exposure to water in which infected snails reside. Many parasitic infections are chronic because of weak innate immunity and the ability of parasites to evade or resist elimination by adaptive immune responses. Furthermore, many antiparasitic drugs are not effective at killing the organisms. Individuals living in endemic areas require repeated chemotherapy because of continued exposure, and such treatment is often not possible because of expense and logistic problems.

Innate Immunity to Parasites

Although different protozoan and helminthic parasites have been shown to activate different mechanisms of innate immunity, these organisms are often able to survive and replicate in their hosts because they are well adapted to resisting host defenses. The principal innate immune response to protozoa is phagocytosis, but many of these parasites are resistant to phagocytic killing and may even replicate within macrophages. Some protozoa express surface molecules that are recognized by TLRs and activate phagocytes. *Plasmodium* species (the protozoa that are responsible for malaria), *Toxoplasma gondii* (the agent that causes toxoplasmosis), and *Cryptosporidium* species (a major cause of diarrheal disease in HIV-infected patients) all express glycolipids that can activate TLR2 and TLR4. Eosinophils contribute to the innate response to helminths by releasing granule contents that are capable of destroying worm integuments. Phagocytes may also attack helminthic parasites and secrete microbicidal substances to kill organisms. However, many helminths have thick integuments that make them resistant to the cytocidal mechanisms of neutrophils and macrophages, and they are too large to be ingested by these phagocytes. Some protozoa and helminths activate the alternative pathway of complement, but they have also developed effective strategies for evading the complement system.

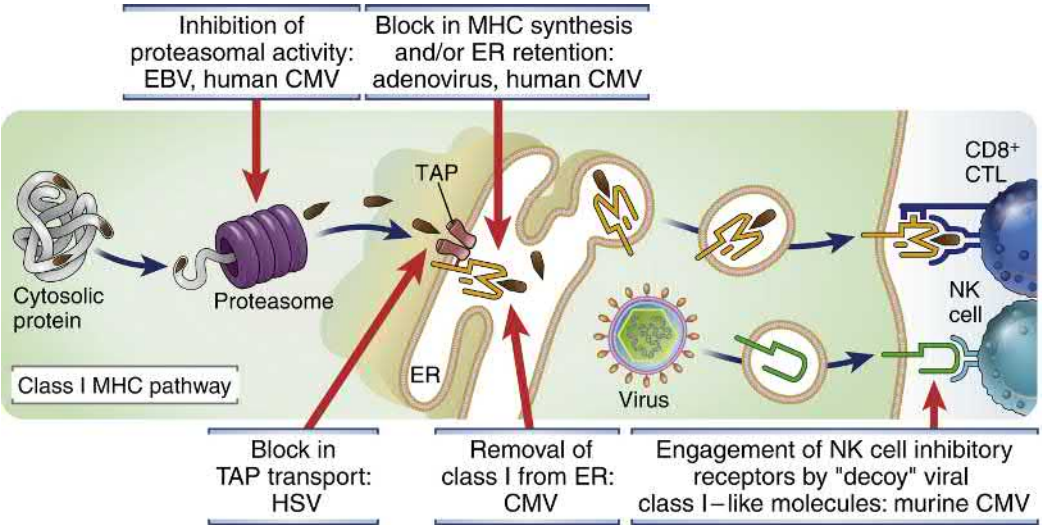

Inhibition of proteasomal activity: EBV, human CMV
Block in MHC synthesis and/or ER retention: adenovirus, human CMV
TAP
CD8+ CTL
Cytosolic protein
Proteasome
NK cell
Class I MHC pathway
ER
Virus
Block in TAP transport: HSV
Removal of class I from ER: CMV
Engagement of NK cell inhibitory receptors by "decoy" viral class I–like molecules: murine CMV

FIGURE 16.11 Mechanisms by which viruses inhibit recognition by $CD8^+$ T cells and NK cells. The pathway of class I major histocompatibility complex *(MHC)*-associated antigen presentation is shown, with examples of viruses that block different steps in this pathway. In addition to interfering with recognition by $CD8^+$ T cells, some viruses produce “decoy” MHC molecules that engage inhibitory receptors of NK cells. *CMV,* Cytomegalovirus; *CTL,* cytotoxic T lymphocyte; *EBV,* Epstein-Barr virus; *ER,* endoplasmic reticulum; *HSV,* herpes simplex virus; *TAP,* transporter associated with antigen processing.

TABLE 16.4

Parasite	Disease	Principal Mechanisms of Protective Immunity
Protozoa		
***Plasmodium* species**	Malaria	Antibodies and $CD8^+$ cytotoxic T lymphocytes
Leishmania donovani	Leishmaniasis (mucocutaneous disseminated)	$CD4^+$ Th1 cells activate macrophages to kill phagocytosed parasites
Trypanosoma brucei	African trypanosomiasis	Antibodies
Entamoeba histolytica	Amebiasis	Antibodies, phagocytosis
Metazoa		
***Schistosoma* species**	Schistosomiasis	Killing by eosinophils, macrophages
Filaria (e.g., *Wuchereria bancrofti*)	Filariasis	Cell-mediated immunity; role of antibodies?

[a] Selected examples of parasites and immune responses to them are listed.

Adaptive Immunity to Parasites

Different protozoa and helminths vary greatly in their structural and biochemical properties, life cycles, and pathogenic mechanisms. It is therefore not surprising that different parasites elicit distinct adaptive immune responses (Table 16.4). Some pathogenic protozoa have evolved to survive within host cells, so protective immunity against these organisms is mediated by mechanisms similar to those that eliminate intracellular bacteria and viruses. In contrast, metazoa such as helminths survive in extracellular tissues, and their elimination often depends on special types of antibody responses.

The principal defense mechanism against protozoa that survive within macrophages is cell-mediated immunity, particularly macrophage activation by Th1 cell–derived cytokines. Infection of mice with *L. major*, a protozoan that survives within the endosomes of macrophages, illustrates how dominance of Th1 or Th2 responses determines disease resistance or susceptibility (Fig. 16.12). Resistance to the infection is associated with activation of leishmania-specific Th1 cells, which produce IFN-γ and thereby activate macrophages to destroy intracellular parasites. Conversely, activation of Th2 cells by the protozoa results in increased parasite survival and exacerbation of lesions because Th2 cytokines inhibit classical macrophage activation. Most inbred strains of mice are resistant to infection with *L. major,* but inbred BALB/c and some related strains of mice are highly susceptible and die if they are infected with high doses of parasites. The resistant strains produce large amounts of IFN-γ in response to leishmanial antigens, whereas the strains that are susceptible to fatal leishmaniasis produce more IL-4 in response to the parasite. Promoting the Th1 response or inhibiting the Th2 response in susceptible strains increases their resistance to the infection. The mechanisms of this striking difference between strains of mice are not defined.

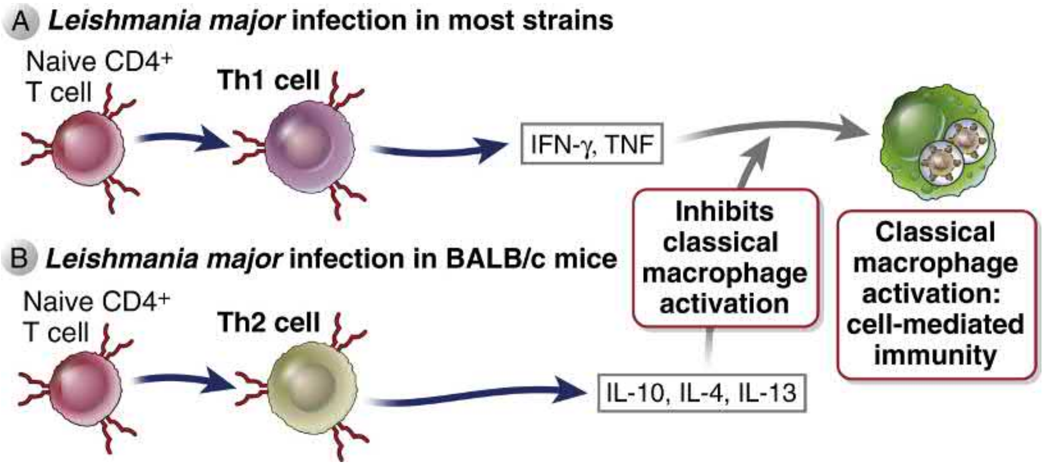

A *Leishmania major* infection in most strains
Naive CD4+ T cell
Th1 cell
IFN-γ, TNF
B *Leishmania major* infection in BALB/c mice
Naive CD4+ T cell
Th2 cell
IL-10, IL-4, IL-13
Inhibits classical macrophage activation
Classical macrophage activation: cell-mediated immunity

FIGURE 16.12 Role of T cells and cytokines in determining the outcome of infections. Naive $CD4^+$ T lymphocytes may differentiate into Th1 cells, which activate phagocytes to kill ingested microbes, and Th2 cells, which inhibit this classical pathway of macrophage activation. The balance between these two T cell subsets may influence the outcome of infections, as illustrated by *Leishmania* infection in mice—most mouse strains develop Th1 responses against the parasite and effectively clear the organisms, but BALB/c mice develop strong Th2 responses and succumb to the infection. *IFN,* Interferon; *IL,* interleukin; *TNF,* tumor necrosis factor.

Protozoa that replicate inside various host cells and lyse these cells stimulate specific antibody and CTL responses, similar to cytopathic viruses. An example of such an organism is the malaria parasite, which resides mainly in red blood cells and in hepatocytes during its life cycle. It was thought for many years that antibodies were the major protective mechanism against malaria, and early attempts at vaccinating against this infection focused on generating protective antibodies. It is now apparent that the CTL response against parasites residing in hepatocytes is an important defense against the spread of this intracellular protozoan. The cytokine IFN-γ has been shown to be protective in many protozoal infections, including malaria, toxoplasmosis, and cryptosporidiosis.

Defense against many helminthic infections is mediated by the activation of Th2 cells, which results in production of IgE antibodies and activation of eosinophils. Helminths stimulate differentiation of naive CD4$^+$ T cells to the Th2 subset of effector cells, which secrete IL-4 and IL-5. IL-4 stimulates the production of IgE, which binds to the Fcε receptor of mast cells, and IL-5 activates eosinophils. The combined actions of mast cells and eosinophils also contribute to expulsion of the parasites from the intestine, although exactly how they contribute remains poorly defined. The expulsion of some intestinal nematodes may be due to Th2–dependent mechanisms that do not require IgE, such as increased mucus production and peristalsis caused by Th2 cytokines.

Adaptive immune responses to parasites can also contribute to tissue injury. Some parasites and their products induce granulomatous responses with concomitant fibrosis. *Schistosoma mansoni* eggs deposited in the liver stimulate CD4$^+$ T cells, which in turn activate macrophages and induce DTH reactions. DTH reactions result in the formation of granulomas around the eggs; an unusual feature of these granulomas, especially in mice, is their association with Th2 responses. (Granulomas are generally induced by Th1 responses against persistent antigens; see Chapter 19.) Such Th2-induced granulomas serve to contain the schistosome eggs, but severe fibrosis associated with this chronic cell-mediated immune response leads to cirrhosis, disruption of venous blood flow in the liver, and portal hypertension. In lymphatic filariasis, lodging of the parasites in lymphatic vessels leads to chronic cell-mediated immune reactions and ultimately to fibrosis. This results in lymphatic obstruction and severe lymphedema. Chronic and persistent parasitic infestations are often associated with the formation of complexes of parasite antigens and specific antibodies. The complexes can be deposited in blood vessels and kidney glomeruli and produce vasculitis and nephritis, respectively (see Chapter 19). Immune complex disease is a complication of schistosomiasis and malaria.

Immune Evasion by Parasites

Parasites evade protective immunity by reducing their immunogenicity and by inhibiting host immune responses. Different parasites have developed remarkably effective ways of resisting immunity (Table 16.5).

TABLE 16.5

Mechanisms of Immune Evasion by Parasites

Mechanism of Immune Evasion	Examples
Antigenic variation	Trypanosomes, *Plasmodium*
Acquired resistance to complement, cytotoxic T lymphocytes	Schistosomes
Inhibition of host immune responses	Filaria (secondary to lymphatic obstruction), trypanosomes
Antigen shedding	*Entamoeba*

- Parasites change their surface antigens during their life cycle in vertebrate hosts. Two forms of antigenic variation are well defined. The first is a stage-specific change in antigen expression, such that the mature tissue stages of parasites produce antigens different from those of the infective stages. For example, the infective sporozoite stage of malaria parasites is antigenically distinct from the merozoites that reside in the host and are responsible for chronic infection. By the time the immune system has responded to infection by sporozoites, the parasite has differentiated, expresses new antigens, and is no longer a target for immune elimination. A more remarkable example of antigenic variation in parasites is the continuous variation of major surface antigens seen in African trypanosomes, such as *Trypanosoma brucei* and *Trypanosoma rhodesiense.* Continuous antigenic variation in trypanosomes is mainly due to changes in expression of the genes encoding the major surface antigen. Infected patients show waves of blood parasitemia, and each wave consists of parasites expressing a surface antigen that is different from the preceding wave. Thus, by the time the host produces antibodies against the parasite, an antigenically different organism has grown out. More than 100 such waves of parasitemia can occur in a single infection. One consequence of antigenic variation in parasites is that it is difficult to effectively vaccinate individuals against these infections.
- Parasites become resistant to immune effector mechanisms during their residence in vertebrate hosts. Perhaps the best examples are schistosome larvae, which travel to the lungs of infected animals and during this migration develop a tegument that is resistant to damage by complement and by CTLs.
- Protozoan parasites may conceal themselves from the immune system either by living inside host cells or by developing cysts that are resistant to immune effectors.

Some helminthic parasites reside in intestinal lumens and are sheltered from cell-mediated immune effector mechanisms. Parasites may also shed their antigenic coats, either spontaneously or after binding specific antibodies. Shedding of antigens renders the parasites resistant to subsequent antibody-mediated attack. *Entamoeba histolytica* is a protozoan parasite that sheds antigens and can also convert to a cyst form in the lumen of the large intestine.

- Parasites inhibit host immune responses by multiple mechanisms. T cell anergy to parasite antigens has been observed in severe schistosomiasis involving the liver and spleen and in filarial infections. The mechanisms of immunologic unresponsiveness in these infections are not well understood. In lymphatic filariasis, infection of lymph nodes with subsequent architectural disruption may contribute to deficient immunity. Some parasites, such as *Leishmania,* stimulate the development of regulatory T cells, which suppress the immune response enough to allow persistence of the parasites. More nonspecific and generalized immunosuppression is observed in malaria and African trypanosomiasis. This immune deficiency has been attributed to the production of immunosuppressive cytokines by activated macrophages and T cells and defects in T cell activation.

The consequences of parasitic infestations for health and economic development are devastating. Attempts to develop effective vaccines against these infections have been actively pursued for many years. Although the progress has been slow, elucidation of the fundamental mechanisms of immune responses to and immune evasion by parasites holds promise for the future.

Strategies for Vaccine Development

The birth of immunology as a science dates from Edward Jenner's successful vaccination against smallpox in 1796. The importance of prophylactic immunization against infectious diseases is best illustrated by the fact that worldwide programs of vaccination have led to the complete or nearly complete eradication of many of these diseases in many countries (see Table 1.1). The fundamental principle of vaccination is to administer a killed or attenuated form of an infectious agent, or a component of a microbe, that does not cause disease but elicits an immune response that provides protection against infection by the live, pathogenic microbe.

The success of vaccination in eradicating infectious disease depends on several properties of the microbes. Vaccines are most effective if the infectious agent does not establish latency, does not undergo antigenic variation, and does not interfere with the host immune response. It is difficult to effectively vaccinate against microbes such as HIV, which establishes latent infection, is highly variable, and inhibits host immunity. Vaccines are also most effective against infections that are limited to human hosts and do not have animal reservoirs.

Most vaccines in use today work by inducing humoral immunity. Antibodies are the only immune mechanism that prevents infections, by neutralizing and clearing microbes before they gain their foothold in the host. The best vaccines are those that stimulate the development of long-lived plasma cells that produce high-affinity antibodies and memory B cells. These aspects of humoral immune responses are best induced in the germinal center reaction (see Chapter 12), which requires help provided by protein antigen–specific CD4$^+$ T follicular helper (Tfh) cells.

There are major challenges in developing effective vaccines against several important infections. The immunologic correlates of protection are often poorly defined. Fundamental questions about how to maximally stimulate durable memory, effective Tfh cells, and long-lived plasma cells remain unresolved. Clinical experience has taught us that the longevity of vaccine-induced protection varies greatly, being lifelong with hepatitis B antigen vaccines and quite short with many others. The reasons for this critical difference are unknown. It is hoped that continuing advances in basic immunology and in methods for analyzing immune responses in humans will lead to answers to these questions that will put vaccine development on a strong foundation of mechanisms and basic understanding.

In the following section, we will summarize the approaches to vaccination that have been tried (Table 16.6) and their major value and limitations.

Attenuated and Inactivated Bacterial and Viral Vaccines

Some of the earliest (first generation) and most effective vaccines are composed of intact microbes that are treated in such a way that they are attenuated or killed so they can no longer cause disease while retaining their immunogenicity. The great advantage of attenuated microbial vaccines is that they elicit many of the innate and adaptive immune responses (both humoral and cell-mediated) that the pathogenic microbe would, and they are therefore the ideal way of inducing protective immunity. Live, attenuated bacteria were first shown by Louis Pasteur to confer specific immunity. The attenuated or killed bacterial vaccines currently in use generally induce limited protection and are effective for only short periods. Live, attenuated viral vaccines are usually more effective; polio, measles, and yellow fever are three good examples. The earliest approach for producing such attenuated viruses was repeated passage in cell culture. More recently, temperature-sensitive and gene deletion mutants have been generated to create attenuated viruses. Viral vaccines often induce long-lasting specific immunity, so immunization of children is sufficient for lifelong protection. The major concern with attenuated viral or bacterial vaccines is safety. The live-attenuated oral polio vaccine has nearly eradicated the disease, but in rare cases the virus in the vaccine is reactivated and itself causes paralytic polio. In fact, the success of worldwide vaccination is creating the problem that the vaccine-induced disease, although rare, could become more frequent than the naturally acquired disease. This potential problem may have to be tackled by reverting to the killed virus vaccine to complete the eradication program.

TABLE 16.6

Vaccine Approaches [a]

Type of Vaccine	Examples
Live attenuated or killed bacteria	Bacillus Calmette-Guérin, cholera
Live attenuated or killed viruses	Polio, influenza, rabies
Subunit (antigen) vaccines	Tetanus toxoid, diphtheria toxoid
Conjugate vaccines	*Haemophilus influenzae*, pneumococcus
Synthetic vaccines	Hepatitis (recombinant proteins)
Viral vectors	Clinical trials of human SARS-CoV-2 spike protein made by human and chimpanzee adenovirus vectors
DNA vaccines	Clinical trials ongoing for several infections
mRNA vaccines	Approved for COVID-19

[a] The table lists selected examples of vaccines in use as of December 2020.

A widely used inactivated vaccine of considerable public health importance is the influenza vaccine. Influenza viruses grown in chicken eggs are used in two types of vaccines. The most common vaccine is a trivalent inactivated (killed) vaccine that is used in the flu shot that is given intramuscularly. Three of the most frequently encountered influenza strains are selected every year and incorporated in this vaccine. A second type of influenza vaccine involves the same three strains, but the vaccine is made up of live attenuated viruses and is used as a nasal spray. Two of the major limitations of current influenza vaccines is that they do not induce broadly neutralizing antibodies that recognize multiple strains of the virus and antibody-mediated protection is short lived.

Purified Antigen (Subunit) Vaccines

These second-generation vaccines were produced to eliminate the safety concerns associated with attenuated microbes. Subunit vaccines are composed of antigens purified from microbes or inactivated toxins and are usually administered with an adjuvant. One effective use of purified antigens as vaccines is for the prevention of diseases caused by bacterial toxins. Toxins can be rendered harmless without loss of immunogenicity, and such toxoids induce strong antibody responses. Diphtheria and tetanus are two infections whose life-threatening consequences have been largely controlled because of immunization of children with toxoid preparations. Vaccines composed of bacterial polysaccharide antigens are used against pneumococcus and *Haemophilus influenzae*. Because polysaccharides are T-independent antigens, they tend to elicit low-affinity antibody responses and are poorly immunogenic in infants (who do not mount strong T cell–independent antibody responses). High-affinity antibody responses may be generated against polysaccharide antigens even in infants by coupling the polysaccharides to proteins to form **conjugate vaccines** (Fig. 16.13). These vaccines elicit helper T cells to simulate germinal center reactions, which would not occur with simple polysaccharide vaccines. Such vaccines work like hapten-carrier conjugates and are a practical application of the principle of T and B cell cooperation (see Chapter 12). The currently used *H. influenzae,* pneumococcal, and meningococcal vaccines are conjugate vaccines. Purified protein vaccines stimulate helper T cells and antibody responses, but they do not generate potent CTLs. The reason for poor CTL development is that exogenous proteins (and peptides) usually enter the class II MHC pathway of antigen presentation (except in the special situation of cross-presentation). As a result, protein vaccines are not recognized efficiently by class I MHC–restricted CD8$^+$ T cells.

Synthetic Antigen Vaccines

A goal of vaccine research has been to identify the most immunogenic microbial antigens or epitopes, to synthesize these in the laboratory, and to use the synthetic antigens as vaccines. It is possible to deduce the protein sequences of microbial antigens from nucleotide sequence data and to prepare large quantities of proteins by recombinant DNA technology. Vaccines made of recombinant DNA–derived antigens are now in use for hepatitis B virus and HPV. In the case of the most widely used HPV vaccine, which was developed to prevent cancers caused by the virus, recombinant viral proteins from four strains (HPV 6, 11, 16, and 18) are made in yeast and combined with an adjuvant. HPV 6 and 11 are common causes of warts, and HPV 16 and 18 are the HPV strains most often linked to cervical cancer.

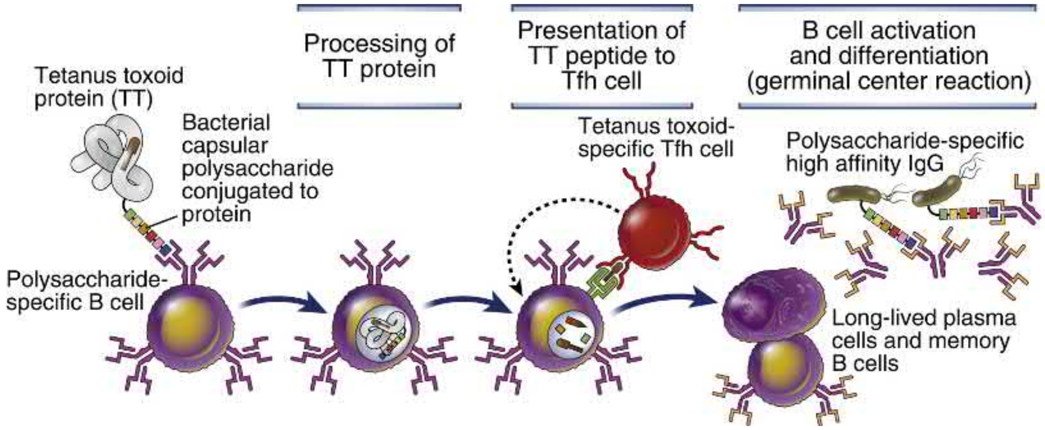

Processing of TT protein
Presentation of TT peptide to Tfh cell
B cell activation and differentiation (germinal center reaction)
Tetanus toxoid protein (TT)
Bacterial capsular polysaccharide conjugated to protein
Tetanus toxoid-specific Tfh cell
Polysaccharide-specific high affinity IgG
Polysaccharide-specific B cell
Long-lived plasma cells and memory B cells

FIGURE 16.13 Conjugate vaccines.Conjugates of bacterial polysaccharides and a protein carrier (in this case, tetanus toxoid *[TT]*) induce potent high-affinity antibody responses because the protein carrier recruits helper T cells into the reaction. Note that the antibody produced is specific for the polysaccharide. *IgG,* Immunoglobulin G; *Tfh,* T follicular helper.

Live Viral Vaccines Involving Recombinant Viruses

Another approach for vaccine development is to introduce genes encoding microbial antigens into a noncytopathic virus and to infect individuals with this virus. Thus, the virus serves as a source of the antigen in an inoculated individual. The great advantage of viral vectors is that they, like other live viruses, induce the full complement of immune responses, including strong CTL responses. This technique has been used most commonly with vaccinia virus vectors, and more recently with canarypox viral vectors, which are not pathogenic in humans. Inoculation of such recombinant viruses into many species of animals induces both humoral and cell-mediated immunity against the antigen produced by the foreign gene (and, of course, against antigens of the viral vectors as well). A potential problem with recombinant viruses is that the viruses may infect host cells, and even though they are not pathogenic, they may produce antigens that stimulate CTL responses that kill the infected host cells. Also, the nonpathogenic virus could recombine with host viruses or gene sequences and become virulent. These safety concerns have limited widespread use of viral vectors for vaccine delivery. One approach that overcomes many of these issues and concerns is the use of live recombinant hybrid vaccines that are non-replicating. An adenovirus 26 vector (humans generally lack antibodies to this adenovirus) and a chimpanzee adenovirus vector have been used to generate vaccines to a number of viruses, including Ebola virus, Zika virus, and SARS-CoV-2. Non-replicating adenoviruses infect numerous host cells and thus produce a significant amount of the viral antigen.

DNA Vaccines

An interesting method of vaccination was developed on the basis of an unexpected observation. Inoculation of a plasmid containing complementary DNA (cDNA) encoding a protein antigen leads to humoral and cell-mediated immune responses to the antigen. It is likely that APCs, such as DCs, are transfected by the plasmid and the cDNA is transcribed and translated into immunogenic protein that elicits specific responses. Bacterial plasmids are rich in unmethylated CpG nucleotides that are recognized by TLR9 in DCs and other cells, thereby eliciting an innate immune response that enhances adaptive immunity (see Chapter 4). Therefore, plasmid DNA vaccines could be effective even when administered without adjuvants. The ability to store DNA without refrigeration for use in the field also makes this technique promising. However, DNA vaccines have not been as effective as hoped in clinical trials, mainly because the first generation of these vaccines did not produce adequate amounts of the immunogen. Studies with newer vectors for DNA vaccination are currently in progress.

mRNA Vaccines

Another relatively recent mode of vaccination uses messenger RNA (mRNA) encoding microbial antigens. The main advantages of mRNA vaccines are the ease with which they can be rapidly developed, the ability to bypass the need for the large-scale manufacture and purification of protein antigens (thereby greatly reducing the cost), and the ability to combine mRNAs encoding many different protein antigens from a pathogen into a single vaccine. Although initial attempts to use mRNA were unsuccessful, largely because of stability issues, a number of recent advances have made mRNA vaccination a practical modality. One major advance is modifications of the mRNA itself. These modifications include addition of a synthetic 5′ cap and a long poly A-tail to increase stability, altering 5′ and 3′ untranslated regions of the mRNA to enhance both translation and stability, and codon optimization of the coding portions to enhance translatability. Current mRNA vaccines for COVID-19 retain some ability to activate innate immunity by triggering RNA sensors. The mRNA is encapsulated in lipid nanoparticles that facilitate uptake by cells, including dendritic cells, and also function as an adjuvant. Another approach to mRNA vaccines, not yet in clinical use, involves linking the mRNA to a modified alphavirus RNA genome that allows for self-replication, thus allowing many copies of the vaccine to be generated in recipient cells.

Adjuvants and Immunomodulators

The initiation of T cell–dependent immune responses against protein antigens requires that the antigens be administered with adjuvants. Most adjuvants elicit innate immune responses, with increased expression of costimulators and production of cytokines, such as IL-12, that stimulate T cell growth and differentiation. Heat-killed bacteria are powerful adjuvants that are commonly used in experimental animals. However, the severe local inflammation that such bacteria trigger precludes their use as adjuvants in humans.

Much effort is currently being devoted to development of safe and effective adjuvants for clinical use. Only a few are approved for patients: aluminum hydroxide gel (which appears to promote mostly B cell responses); a bacterial product, monophosphoryl lipid A, alone or with aluminum salt; and a lipid formulation called squalene that may activate phagocytes. Recently, CG-rich oligonucleotides (CpG DNA) have been approved as an adjuvant for hepatitis B vaccines; by activating TLR9, these agents elicit potent innate immune reactions. An alternative to adjuvants is to administer natural substances that stimulate T cell responses together with antigens. As mentioned, plasmid DNA and some mRNA formulations have intrinsic adjuvant-like activities, and it is possible to incorporate costimulators (e.g., B7 molecules) or cytokines into plasmid DNA vaccines. These interesting ideas remain experimental.

Passive Immunization

Protective immunity also can be conferred by passive immunization, for instance, by transfer of specific antibodies. In the clinical situation, passive immunization is most commonly used for rapid treatment of potentially fatal diseases caused by toxins, such as tetanus, and for protection from rabies, hepatitis, and SARS-CoV-2. Antibodies against snake venom can be lifesaving when administered after poisonous snakebites. Convalescent plasma has been used in cases of Ebola and COVID-19. Recombinant monoclonal neutralizing antibodies are now utilized as a therapy for COVID-19. Passive immunity, using current approaches, is short-lived because the host does not respond to the immunization, and protection lasts only as long as the injected antibody persists. Moreover, passive immunization does not induce memory, so an immunized individual is not protected against subsequent exposure to the toxin or microbe. However, based on the successful identification of human broadly neutralizing monoclonal antibodies against pathogens, such as HIV and the flu virus, newer attempts for long-term passive immunization using a process called vectored immunoprophylaxis have been developed. In this approach, adeno-associated viral vectors are used to introduce cloned human Ig heavy and light chain genes for a neutralizing antibody into human subjects. The goal is to have injected humans synthesize a specific protective broadly neutralizing antibody for an extended period. Clinical trials have been initiated.

Summary

- The interaction of the immune system with infectious organisms is a dynamic interplay of host mechanisms aimed at eliminating infections and microbial strategies designed to permit survival in the face of powerful defenses. Different types of infectious agents stimulate distinct types of immune responses and have evolved unique mechanisms for evading immunity. In some infections, the immune response is the cause of tissue injury and disease.
- Innate immunity against extracellular bacteria is mediated by phagocytes and the complement system (the alternative and lectin pathways).
- The principal adaptive immune response against extracellular bacteria consists of specific antibodies that opsonize the bacteria for phagocytosis and activate the complement system. Toxins produced by such bacteria are neutralized by specific antibodies. Some bacterial toxins are powerful inducers of cytokine production, and cytokines account for much of the systemic disease associated with severe, disseminated infections with these microbes.
- Innate immunity against intracellular bacteria is mediated mainly by macrophages. However, intracellular bacteria are capable of surviving and replicating within host cells, including phagocytes, because they have developed mechanisms for resisting degradation within phagocytes.

- Adaptive immunity against intracellular bacteria is principally cell-mediated and consists of activation of macrophages by CD4$^+$ T cells, as well as killing of infected cells by CD8$^+$ cytotoxic T lymphocytes (CTLs). The characteristic pathologic response to infection by intracellular bacteria is granulomatous inflammation.
- Protective responses to fungi consist of innate immunity, mediated by neutrophils and macrophages; adaptive cell-mediated immunity, mainly involving Th17 cells; and humoral immunity. Fungi are usually readily eliminated by phagocytes, because of which disseminated fungal infections are seen mostly in immunodeficient persons.
- Innate immunity against viruses is mediated by type I interferons and natural killer cells. Neutralizing antibodies protect against virus entry into cells early in the course of infection and later if the viruses are released from killed infected cells. The major defense mechanism against established infection is CTL-mediated killing of infected cells. CTLs may contribute to tissue injury even when the infectious virus is not harmful by itself. Viruses evade immune responses involving by antigenic variation, blocking type I IFN production or action, inhibition of antigen presentation, inactivation of T cells, and production of immunosuppressive molecules.
- Parasites such as protozoa and helminths give rise to chronic and persistent infections because innate immunity against them is weak and parasites have evolved multiple mechanisms for evading and resisting specific immunity. The structural and antigenic diversity of pathogenic parasites is reflected in the heterogeneity of the adaptive immune responses that they elicit. Protozoa that live within host cells are destroyed by cell-mediated immunity, whereas helminths are eliminated by eosinophil-mediated killing. Parasites evade the immune system by varying their antigens during residence in vertebrate hosts, by acquiring

resistance to immune effector mechanisms, and by masking and shedding their surface antigens.

- Vaccination is a powerful strategy for preventing infections. The most effective vaccines are those that stimulate the production of high-affinity antibodies and memory cells. Many approaches for vaccination are in clinical use and being tried for various infections.

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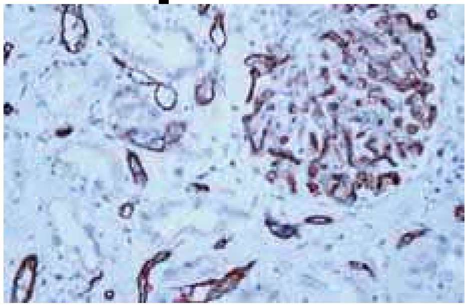
Chapter 17: Transplantation Immunology



General Principles of Transplantation Immunology,

Immune Responses to Allografts,
  The Nature of Alloantigens,
  Recognition of Alloantigens by T Cells,
  Activation and Effector Functions of Alloreactive T Lymphocytes,
  Activation of Alloreactive B Cells and Production and Functions of Alloantibodies,
  Innate Immune Responses to Allografts,

Patterns and Mechanisms of Allograft Rejection,
  Hyperacute Rejection,
  Acute Rejection,
  Chronic Rejection,

Prevention and Treatment of Allograft Rejection,
  Methods to Reduce the Immunogenicity of Allografts,

  - Immunosuppression to Prevent or to Treat Allograft Rejection,
  - Methods to Induce Donor-Specific Tolerance,
- Xenogeneic Transplantation,
- Blood Transfusion and the Abo and Rh Blood Group Antigens,
  - ABO Blood Group Antigens,
  - Other Blood Group Antigens,
- Hematopoietic Stem Cell (HSC) Transplantation,
  - Indications, Methods, and Immune Barriers in Hematopoietic Stem Cell Transplantation,
  - Immunologic Complication of Hematopoietic Stem Cell Transplantation,
- Summary,

Transplantation is widely used for replacing nonfunctioning organs and tissues with healthy organs or tissues. Transplantation is the process of taking cells, tissues, or organs, called a **graft,** from one individual and placing them into a (usually) different individual. The individual who provides the graft is called the **donor,** and the individual who receives the graft is called either the **recipient** or the **host.** If the graft is placed into its normal anatomic location, the procedure is called orthotopic transplantation; if the graft is placed in a different site, the procedure is called heterotopic transplantation. **Transfusion** refers to the transfer of circulating blood cells or plasma from one individual to another. Clinical transplantation to treat human diseases has increased steadily during the past 60 years. Transplantation of hematopoietic stem cells (HSCs), kidneys, livers, and hearts is now common practice in clinical medicine, and transplantation of other organs such as lung and pancreas is becoming more frequent (Fig. 17.1). Almost 40,000 kidney, heart, lung, liver, pancreas, and intestine transplants are currently performed in the United States each year. Transplantation of hands and faces are also now performed in a few medical centers, and transplantation of many other organs or cells, including tissue stem cells, is being attempted.

After the technical challenge of surgically transplanting organs was overcome, it soon became clear that the immune response against grafted tissues is the major barrier to survival of transplanted tissues or organs. Conversely, controlling this immune response is key to successful transplantation. These realizations have led to the development of transplantation immunology as a discipline within the broader topic of immunology, and this is the theme of this chapter.

General Principles of Transplantation Immunology

Based on experimental studies and clinical observations, there are several principles that uniquely apply to immune responses against transplants.

Transplantation of cells or tissues from one individual to a genetically nonidentical individual invariably leads to rejection of the transplant because of an adaptive immune response. This problem was first appreciated when attempts to replace damaged skin on burn patients with skin from unrelated donors proved to be uniformly unsuccessful. Within 1 to 2 weeks, the transplanted skin would undergo necrosis and fall off. The failure of the grafts led Peter Medawar and other investigators to study skin transplantation in animal models. These experiments established that the failure of skin grafting was caused by an inflammatory reaction, which was called **rejection.** The knowledge that graft rejection is the result of an adaptive immune response came from experiments demonstrating that the process had characteristics of memory and specificity and was mediated by lymphocytes (Fig. 17.2). For instance, rejection occurs 10 to 14 days after the first transplant from a donor to a nonidentical recipient (called first-set rejection) and more rapidly after the second transplant from the same donor to this recipient (called second-set rejection), implying that the recipient developed memory for the grafted tissue. Individuals who have rejected a graft from one donor show accelerated rejection of another graft from the same donor but not from a different donor, demonstrating that the rejection process is immunologically specific. These experimental results were recapitulated in clinical transplantation. Perhaps the most compelling evidence showing that allograft rejection is an adaptive immune response was the finding that the ability to rapidly reject a transplant with second-set kinetics can be transferred with lymphocytes from a transplant recipient animal to a naive animal.

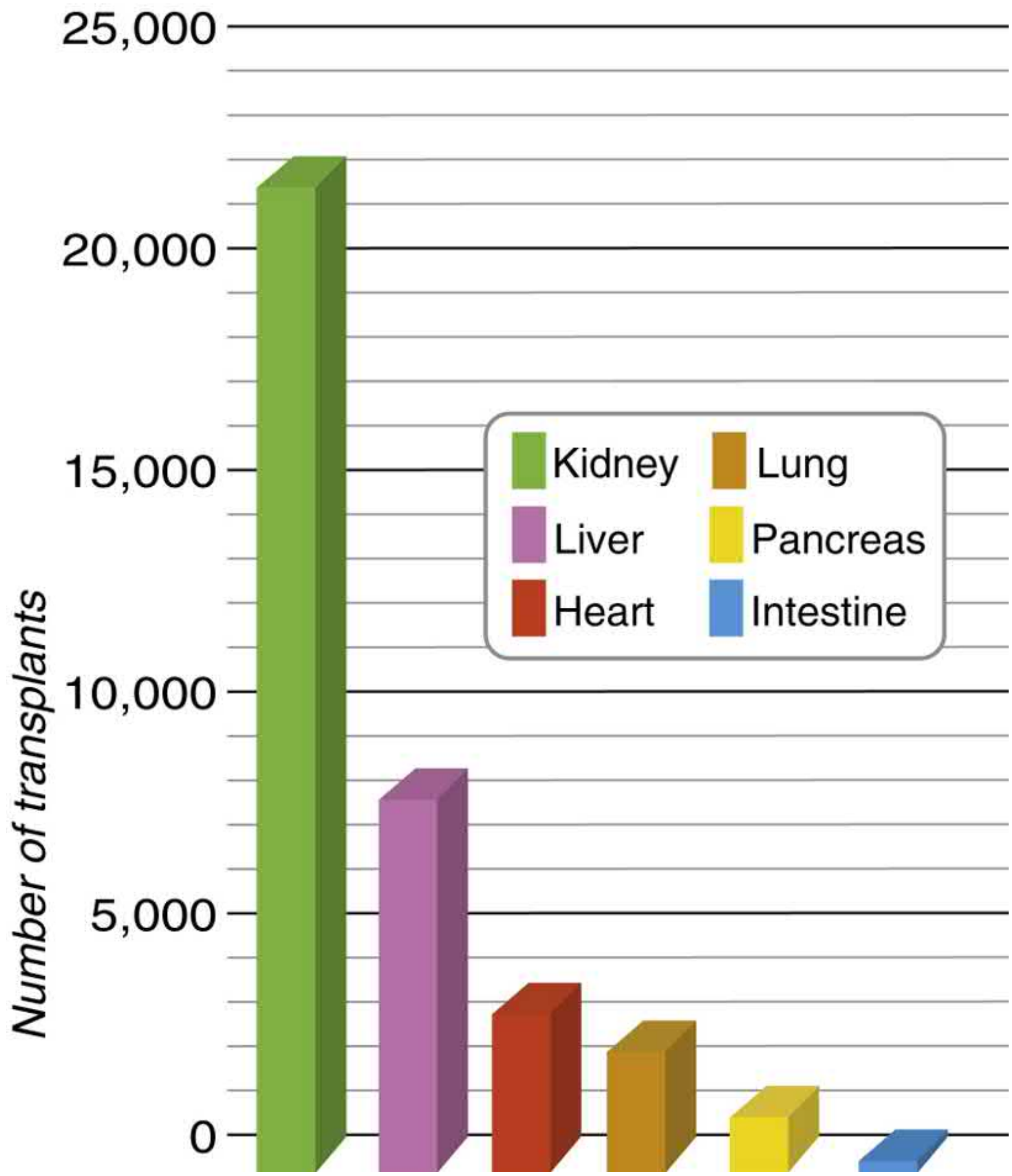


FIGURE 17.1 Number of solid organ transplants in the United States in 2018 by organ type.

Data from United Network for Organ Sharing. Available at https://unos.org/data/transplant/.

Transplant immunologists have developed a special vocabulary to describe the kinds of cells and tissues encountered in the transplant setting. A graft transplanted from one individual to the same individual is called an **autologous graft.** A graft transplanted between two genetically identical individuals is called a **syngeneic**

graft. A graft transplanted between two genetically different individuals of the same species is called an **allogeneic graft** (or **allograft**). A graft transplanted between individuals of different species is called a **xenogeneic graft** (or **xenograft**). The molecules that are recognized as foreign in allografts are called **alloantigens,** and those in xenografts are called **xenoantigens.** The lymphocytes and antibodies that react with alloantigens or xenoantigens are described as being **alloreactive** or **xenoreactive,** respectively.

Most of this chapter focuses on allogeneic transplantation because it is far more commonly practiced than xenogeneic transplantation, which is discussed briefly at the end of the chapter. We will consider both the basic immunology and some aspects of the clinical practice of transplantation. We will conclude the chapter with a discussion of HSC transplantation, which raises special issues not usually encountered with solid organ transplants.

Immune Responses to Allografts

Immune responses that mediate allograft rejection are fundamentally similar to responses that fight microbes. The main difference is the nature of what is recognized as foreign. Adaptive immune responses play the major role in graft rejection, but innate responses may contribute in some situations. In this section, we focus on adaptive immune responses to alloantigens and mention the possible role of innate immunity later.

Alloantigens elicit both cellular and humoral immune responses. The mechanisms of allorecognition are best understood by considering the graft antigens that stimulate allogeneic responses and the properties of the responding lymphocytes.

The Nature of Alloantigens

Most of the antigens that stimulate adaptive immune responses against allografts are proteins encoded by polymorphic genes that differ among individuals. These proteins are called histocompatibility molecules because they determine if the grafted tissue (*histo,* tissue) is compatible or incompatible with the host's immune system. The most important of these molecules are the major histocompatibility complex (MHC) proteins (see Chapter 6). All of the animals of an inbred strain are genetically identical, and they are homozygous for all genes (except genes on the sex chromosomes in males). In contrast, inbred animals of different strains, and individuals in an outbred species (except identical twins), differ in many of the genes they inherit. The basic rules of transplantation immunology, which were first established from experiments done with genetically defined mice, include the following (Fig. 17.3):

- Cells or organs transplanted between genetically identical individuals (identical twins or members of the same inbred strain of animals) are not rejected.
- Cells or organs transplanted between genetically nonidentical people or members of two different inbred strains of a species are almost always rejected.

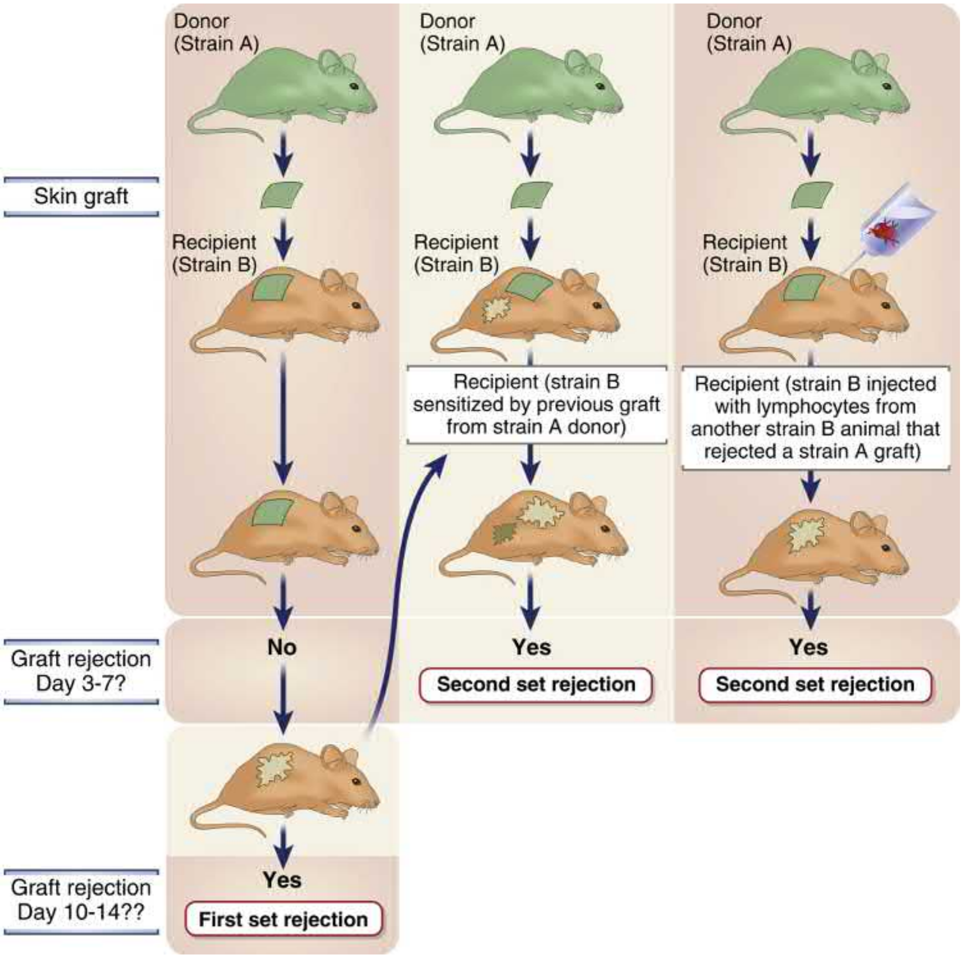

Donor
(Strain A)
Donor
(Strain A)
Donor
(Strain A)
Skin graft
Recipient
(Strain B)
Recipient
(Strain B)
Recipient
(Strain B)
Recipient (strain B
sensitized by previous graft
from strain A donor)
Recipient (strain B injected
with lymphocytes from
another strain B animal that
rejected a strain A graft)
Graft rejection
Day 3-7?
No
Yes
Second set rejection
Yes
Second set rejection
Graft rejection
Day 10-14??
Yes
First set rejection

FIGURE 17.2 First-set and second-set allograft rejection.Results of the experiments shown indicate that graft rejection displays the features of adaptive immune responses, namely memory and mediation by lymphocytes. An inbred strain B mouse will reject a graft from an inbred strain A mouse with first-set kinetics *(left panel)*. An inbred strain B mouse sensitized by a previous graft from an inbred strain A mouse will reject a second graft from an inbred strain A mouse with second-set kinetics *(middle panel),* demonstrating memory. An inbred strain B mouse injected with lymphocytes from another strain B mouse that has rejected a

graft from a strain A mouse will reject a graft from a strain A mouse with second-set kinetics *(right panel),* demonstrating the role of lymphocytes in mediating rejection and memory. An inbred strain B mouse sensitized by a previous graft from a strain A mouse will reject a graft from a third unrelated strain with first-set kinetics, thus demonstrating another feature of adaptive immunity, specificity *(not shown)*. Syngeneic grafts are never rejected *(not shown).*

- The offspring of a mating between two different inbred strains of animal will not reject grafts from either parent. In other words, an (A × B) F1 animal will not reject grafts from an A or B strain animal. (This rule is violated by HSC transplantation, when natural killer (NK) cells in an (A × B) F1 recipient do reject HSCs from either parent, as we will discuss later in this chapter.)
- A graft derived from the offspring of a mating between two different inbred strains of animal will be rejected by either parent. In other words, a graft from an (A × B) F1 animal will be rejected by either an A or a B strain animal.

Such results indicated that the molecules in the grafts that are responsible for eliciting rejection must be polymorphic and their expression is codominant. Polymorphic refers to the property that these graft antigens differ among the individuals of a species (other than identical twins) or between different inbred strains of animals. Codominant expression means that every individual inherits genes encoding these molecules from both parents, and both parental alleles are expressed. Therefore, (A × B) F1 animals express both A and B alleles, see both A and B tissues as self, and are tolerant to both A and B proteins. By contrast, inbred A or B animals express only that allele, are not tolerant to the proteins they do not express, and see (A × B) F1 tissues as partly foreign. Thus, an (A × B) F1 animal does not reject either A or B strain grafts whereas both A and B strain recipients reject an (A × B) F1 graft.

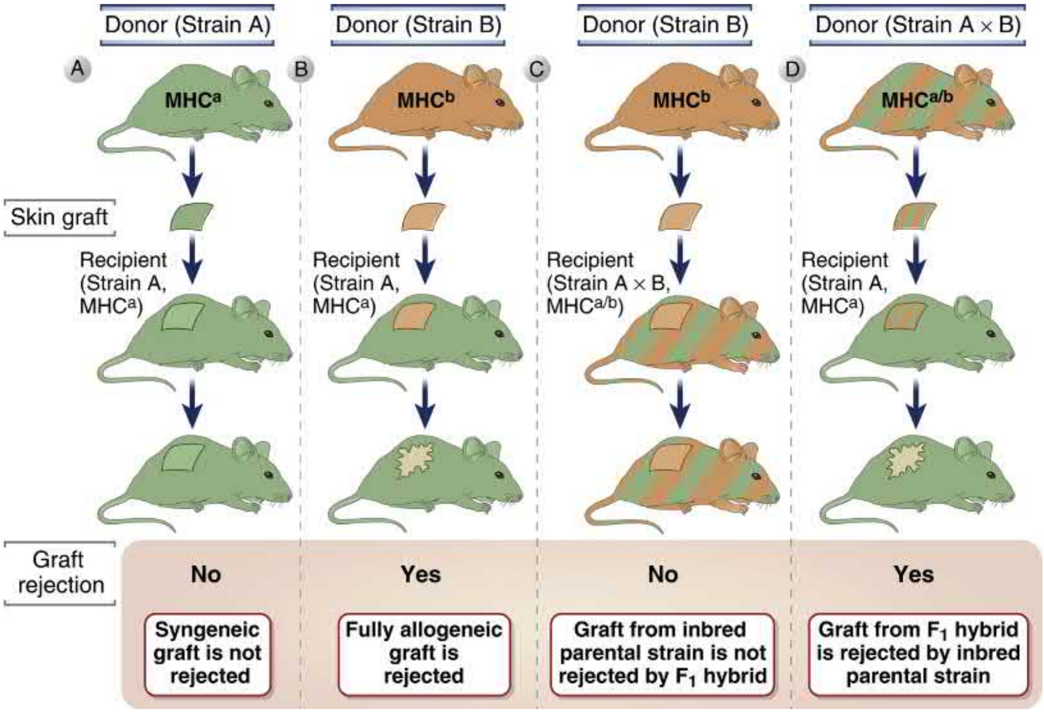

Donor (Strain A)
Donor (Strain B)
Donor (Strain B)
Donor (Strain A × B)
A
B
C
D
MHCa
MHCb
MHCb
MHCa/b
Skin graft
Recipient (Strain A, MHCa)
Recipient (Strain A, MHCa)
Recipient (Strain A × B, MHCa/b)
Recipient (Strain A, MHCa)
Graft rejection
No
Yes
No
Yes
Syngeneic graft is not rejected
Fully allogeneic graft is rejected
Graft from inbred parental strain is not rejected by F1 hybrid
Graft from F1 hybrid is rejected by inbred parental strain

FIGURE 17.3 The genetics of graft rejection.In the illustration, the two different mouse colors represent inbred strains with different sets of genes, referred to here as A or B, encoding molecules important for graft rejection, called major histocompatibility complex *(MHC)* molecules. Inherited MHC alleles from both parents are codominantly expressed in the skin of an A × B offspring, and therefore these mice are represented by both colors. Syngeneic grafts are not rejected **(A)**. Allografts are always rejected **(B)**. Grafts from an A or a B parent will not be rejected by an (A × B) F1 offspring **(C)**, but grafts from the F1 will be rejected by either parent **(D).** These phenomena are due to the fact that *MHC* gene products are responsible for graft rejection; grafts are rejected only if they express an MHC type (represented by *green* or *orange*) that is not expressed by the recipient mouse.

The genetics of graft rejection provided one of the earliest lines of evidence that the adaptive immune system distinguishes self from foreign antigens. All individuals are normally tolerant to self antigens and usually do not react against these, but they do react against foreign antigens. This is now known to be a fundamental property of the normal immune system, where foreign most often refers to microbial antigens. In the context of organ transplantation, foreign usually means products of polymorphic MHC genes.

The molecules responsible for strong and rapid rejection reactions are MHC molecules that bind and present peptides to T cells. MHC molecules, described in Chapter 6, were named before their physiologic function was understood. George Snell and colleagues produced pairs of congenic strains of inbred mice that were bred to be genetically identical to each other except for genes needed for graft rejection. They used these mice to identify the polymorphic genes, which were called MHC genes, that encode the molecular targets of allograft rejection. Transplants of most tissues between any pair of unrelated individuals will be rejected because the genes encoding MHC molecules are so polymorphic that two unrelated individuals are extremely unlikely to inherit the same alleles. The role of MHC molecules as the antigens that cause graft rejection is a consequence of the nature of T cell antigen recognition, as we will discuss later. Recall that human MHC molecules are called human leukocyte antigens (HLA), and in the context of human transplantation, the terms MHC and HLA are used interchangeably.

In any transplant between genetically nonidentical donor and recipient, there will be polymorphic antigens other than MHC molecules against which the recipient may mount an immune response. These antigens typically induce weak or slower (more gradual) rejection reactions than do MHC molecules and are therefore called **minor histocompatibility antigens.** The relevance of minor histocompatibility antigens in clinical solid organ transplantation is uncertain, mainly because there has been little success in identifying the relevant antigens. In mice, the male H-Y antigen appears to be a target of immune recognition by female recipients of grafts from male donors. Although in humans there is a slightly higher risk for rejection of heart transplants from male donor to female recipient, compared with gender-matched transplants, given the scarcity of donor hearts, gender matching is not practical. Minor histocompatibility antigens play a more significant role in stimulating graft-versus-host responses after HSC transplantation, discussed later, but the nature of the relevant antigens in that setting is also not defined.

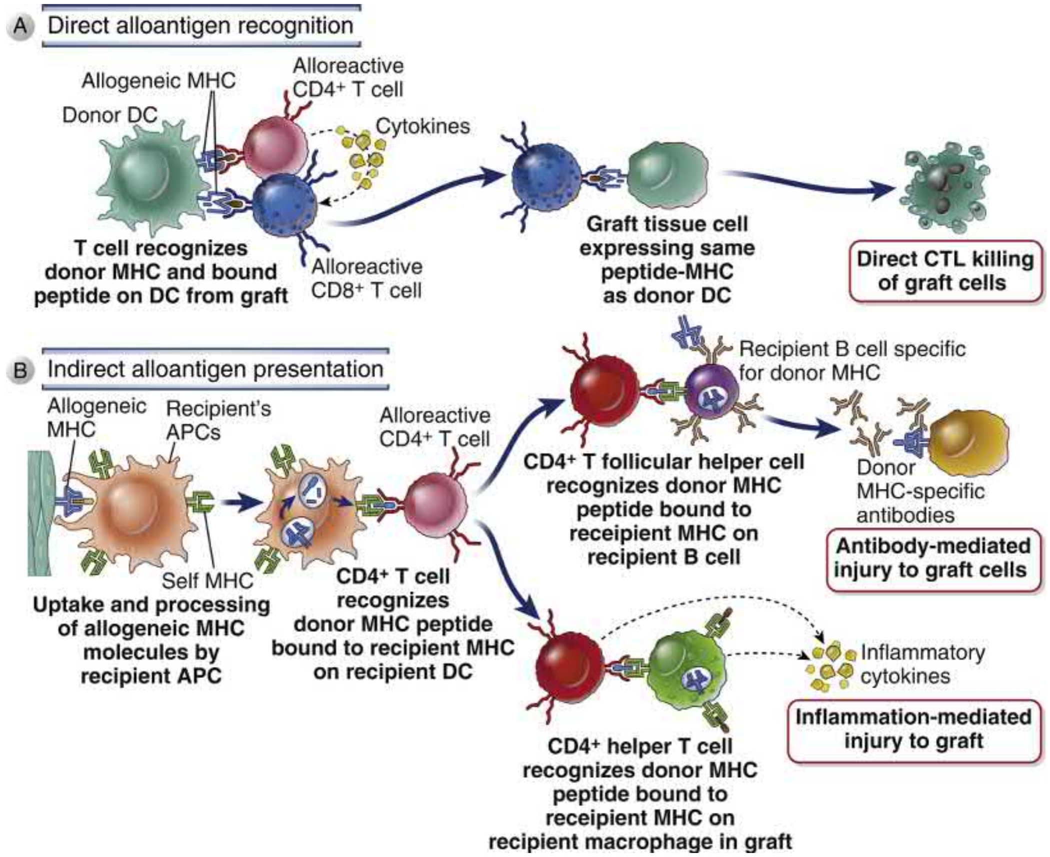

A
Direct alloantigen recognition
Allogeneic MHC
Alloreactive CD4$^+$ T cell
Donor DC
Cytokines
T cell recognizes donor MHC and bound peptide on DC from graft
Alloreactive CD8$^+$ T cell
Graft tissue cell expressing same peptide-MHC as donor DC
Direct CTL killing of graft cells
B
Indirect alloantigen presentation
Allogeneic MHC
Recipient's APCs
Alloreactive CD4$^+$ T cell
Recipient B cell specific for donor MHC
CD4$^+$ T follicular helper cell recognizes donor MHC peptide bound to receipient MHC on recipient B cell
Donor MHC-specific antibodies
Antibody-mediated injury to graft cells
Self MHC
Uptake and processing of allogeneic MHC molecules by recipient APC
CD4$^+$ T cell recognizes donor MHC peptide bound to recipient MHC on recipient DC
Inflammatory cytokines
Inflammation-mediated injury to graft
CD4$^+$ helper T cell recognizes donor MHC peptide bound to receipient MHC on recipient macrophage in graft

FIGURE 17.4 Direct and indirect alloantigen recognition. **A,** Direct alloantigen recognition occurs when alloreactive T cells bind directly to an intact allogeneic major histocompatibility complex *(MHC)* molecule with bound peptide on a graft (donor) dendritic cell *(DC)* or other antigen-presenting cell *(APC),* within lymph nodes. Recipient CD4$^+$ or CD8$^+$ T cells can directly recognize donor class II or class I MHC molecules, respectively, and will differentiate into helper T cells or cytotoxic T lymphocytes *(CTLs).* The CTLs will directly recognize the same donor MHC-peptide complex displayed on graft tissue cells and kill these cells. **B,** Indirect alloantigen recognition occurs when allogeneic MHC molecules from graft cells are taken up and processed by recipient APCs and peptide

fragments of the allogeneic MHC molecules containing polymorphic amino acid residues are bound and presented by recipient (self) MHC molecules. Donor MHC–specific helper T cells that are generated in this way can help B cells to produce donor MHC–specific antibodies that can damage graft cells. The helper T cells also can be activated in the graft by recipient macrophages presenting the same donor MHC–derived peptides, leading to inflammatory damage to the graft.

Recognition of Alloantigens by T Cells

Allogeneic MHC molecules of a graft can be presented for recognition by the recipient's T cells in two different ways, called direct and indirect (Fig. 17.4) **.** Initial studies showed that the T cells of a graft recipient recognize intact, unprocessed MHC molecules in the graft, and this is called **direct presentation** (or **direct recognition**) **of alloantigens.** Subsequent studies showed that sometimes the recipient T cells recognize graft (donor) MHC molecules only in the context of the recipient's MHC molecules, implying that the recipient's MHC molecules must be presenting peptides derived from allogeneic donor MHC proteins to recipient T cells. This process is called **indirect presentation** (or **indirect recognition**), and it is essentially the same as the recognition of any foreign (e.g., microbial) protein antigen. The initial T cell response to MHC alloantigens, whether it results from direct or indirect recognition, most likely occurs in lymph nodes draining the graft, as we will discuss later.

Direct Recognition of Major Histocompatibility Complex Alloantigens on Donor Cells

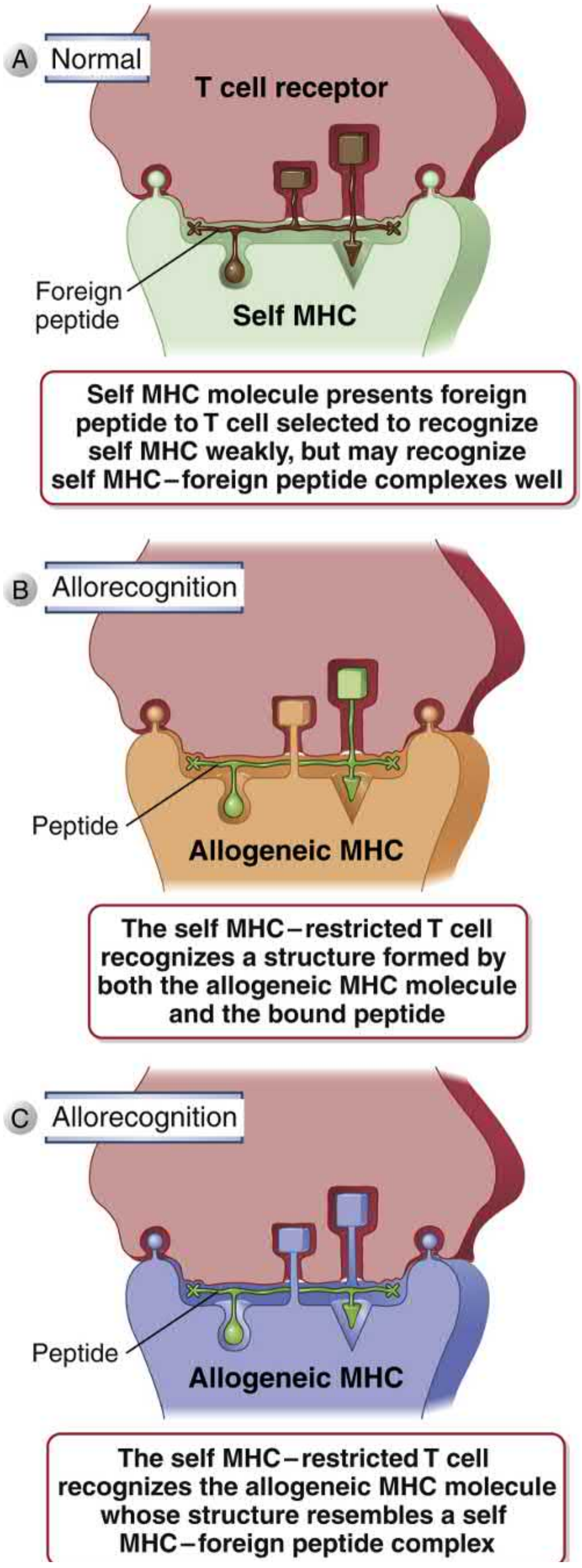
In the case of direct recognition, intact MHC molecules displayed by cells in the graft are recognized by recipient T cells without a need

for processing by host antigen-presenting cells (APCs) (see Fig. 17.4A) **.** It may seem puzzling that T cells that are normally selected during their maturation to be self MHC restricted are capable of recognizing foreign (allogeneic or xenogeneic) MHC molecules. A likely explanation is that T cell receptors (TCRs) have some intrinsic affinity for MHC molecules, regardless of whether they are self or foreign. Furthermore, during T cell development in the thymus, positive selection promotes survival of T cells with weak self MHC reactivity, and among these T cells, there may be many with strong reactivity to allogeneic MHC molecules. Negative selection in the thymus efficiently eliminates T cells with high affinity for self MHC (see Chapters 8 and 15), but it will not eliminate T cells that bind strongly to allogeneic MHC molecules, simply because allogenic MHC molecules are not present in the thymus. The result is that the mature repertoire includes many T cells that bind allogeneic MHC molecules with high affinity. One can think of direct allorecognition as an example of an immunologic cross-reaction in which a T cell that was selected to be self MHC restricted is able to bind structurally similar allogeneic MHC molecules with sufficiently high affinity to permit activation of the T cell (Fig. 17.5).

MHC molecules that are expressed on cell surfaces normally contain bound peptides, and in some cases the peptide contributes to the structure recognized by the alloreactive T cell, exactly like the role of peptides in the normal recognition of foreign antigens by self MHC–restricted T cells (Fig. 17.5B). Even though these peptides may be derived from proteins that are present in both donor and recipient, on the graft cells they are displayed by allogeneic MHC molecules. Therefore, the complexes of peptides (self or foreign) with allogeneic MHC molecules will appear different from self peptide–self MHC complexes. In other cases, direct recognition and activation of an alloreactive T cell may occur regardless of which peptide is carried by the allogeneic MHC molecule, because the polymorphic amino acid residues of the allogeneic MHC molecule alone form a structure that resembles self MHC plus peptide (Fig. 17.5C).

T cell responses to directly presented allogeneic MHC molecules are very strong because there is a high frequency of T cells that can directly recognize any single allogeneic MHC protein. It is estimated that as many as 1% to 10% of all T cells in an individual will directly recognize and react against an allogeneic MHC molecule on a donor cell. In striking contrast, in an infection, the frequency of naive T cells that react against any microbial peptide displayed by self MHC molecules is approximately 1 in 10^5 or 10^6 T cells. There are several explanations for the high frequency of alloreactive T cells.

- Many different peptides derived from donor cellular proteins may combine with a single allogeneic MHC molecule, and each of these peptide-MHC combinations can theoretically activate a different clone of recipient T cells. In contrast, most microbes or protein antigens contain relatively few immunodominant peptides that can be displayed by the self MHC molecules of an individual at any time, so few T cell clones are activated. It is estimated that each APC expresses thousands of MHC molecules. On an allogenic APC, most of these can be recognized by alloreactive T cells at one time. However, in the case of an infection, less than 1% (and perhaps as few as 0.1%) of the self MHC molecules on an APC normally present peptides from that microbe at one time, and only these can be recognized by T cells specific for the microbial antigen.


A Normal
T cell receptor
Foreign peptide
Self MHC

Self MHC molecule presents foreign peptide to T cell selected to recognize self MHC weakly, but may recognize self MHC–foreign peptide complexes well

B Allorecognition
Peptide
Allogeneic MHC

The self MHC–restricted T cell recognizes a structure formed by both the allogeneic MHC molecule and the bound peptide

C Allorecognition
Peptide
Allogeneic MHC

The self MHC–restricted T cell recognizes the allogeneic MHC molecule whose structure resembles a self MHC–foreign peptide complex

FIGURE 17.5 Molecular basis of direct recognition of allogeneic major histocompatibility complex molecules. Direct recognition of allogeneic major histocompatibility complex *(MHC)* molecules may be thought of as a cross-reaction in which a T cell–specific for a self MHC molecule–foreign peptide complex **(A)** also recognizes an allogeneic MHC molecule (**B** and **C**). Donor or self (recipient) peptides that bind to MHC molecules in the graft may contribute to allorecognition **(B)** or they may not **(C).**

- Allogeneic MHC molecules can display not only foreign peptides from donor cells but also self peptides, and these self peptide–foreign MHC complexes can activate T cells. Because these complexes are not normally expressed in the thymus or peripheral tissues, they have not participated in negative selection of T cells that could recognize allogeneic graft cells and potentially cause graft damage. In contrast, T cells specific for self peptides displayed by self MHC molecules are eliminated by negative selection in the thymus and by peripheral tolerance mechanisms (see Chapters 8 and 15). Therefore, the range of peptide-MHC complexes that can activate T cells is much greater if the MHC is allogeneic.
- Many of the T cells that respond to an allogeneic MHC molecule, even on first exposure, are memory T cells. It is likely that these memory cells were generated during previous exposure to other foreign (e.g., microbial) antigens and cross-react with allogeneic MHC molecules. These memory cells not only are expanded populations of antigen-specific cells but also are more rapid and powerful responders than are naive lymphocytes. Thus, these

alloreactive memory T cells contribute to the greater strength of the initial T cell response to a new allograft, compared to the response to the first encounter with a microbial antigen.

Direct allorecognition can generate both $CD4^+$ and $CD8^+$ T cells that recognize graft antigens and contribute to rejection. The role of the alloreactive T cell response in rejection is described later.

Indirect Recognition of Alloantigens

In the indirect pathway, donor (allogeneic) MHC molecules are captured and processed by recipient APCs, and peptides derived from the allogeneic MHC molecules are presented in association with self MHC molecules (see Fig. 17.4B) **.** Thus, peptides from the allogeneic MHC molecules are displayed by host APCs and recognized by T cells like conventional microbial protein antigens. Because allogeneic MHC molecules have amino acid sequences different from those of the host, they themselves can serve as foreign antigens and generate foreign peptides associated with self MHC molecules on the surface of host APCs. Each allogeneic MHC molecule may give rise to multiple peptides that are foreign for the host, each recognized by a different clone of T cells. Indirect presentation may result in allorecognition by $CD4^+$ T cells because alloantigens are acquired by host APCs primarily through the endosomal vesicular pathway (i.e., as a consequence of phagocytosis) and are therefore presented by class II MHC molecules. Some antigens of phagocytosed graft cells do enter the class I MHC pathway of antigen presentation and are indirectly recognized by $CD8^+$ T cells. This phenomenon is an example of cross-presentation or cross-priming (see Fig. 6.14), in which dendritic cells (DCs) ingest proteins of another cell such as from the graft; the proteins are delivered to the cytosol, where they are processed into peptides by proteasomes; and the peptides are presented on class I MHC molecules to activate (prime) $CD8^+$ T lymphocytes.

Evidence that indirect recognition of allogeneic MHC molecules plays a significant role in graft rejection comes from studies with knockout mice lacking class II MHC expression. For example, skin grafts from donor mice lacking class II MHC are able to induce recipient $CD4^+$ (i.e., class II MHC–restricted) T cell responses to peptides derived from donor class I MHC molecules. In these experiments, the donor class I MHC molecules are processed and presented by self class II molecules on the recipient's APCs and stimulate the recipient's helper T cells. Evidence also has been obtained that indirect antigen presentation may contribute to chronic rejection of human allografts (discussed later). $CD4^+$ T cells from heart and liver allograft recipients recognize and are activated by peptides derived from donor MHC when presented by the patient's own APCs. The indirect pathway may become more important over time after transplantation, as donor DCs in the graft are replaced by host DCs.

Activation and Effector Functions of Alloreactive T Lymphocytes

When lymphocytes recognize alloantigens, they become activated to proliferate, differentiate, and perform effector functions that can damage grafts. The activation steps are similar to those we have described for lymphocytes reacting to microbial antigens.

Activation of Alloreactive T Lymphocytes

The T cell response to an organ graft may be initiated in the lymph nodes that drain the graft (Fig. 17.6) **.** Most organs contain resident APCs, such as DCs, and therefore transplanted organs carry with them APCs that express donor MHC molecules. These donor APCs can migrate to regional lymph nodes and present, on their surface, unprocessed allogeneic class I or class II MHC molecules to the recipient's $CD8^+$ and $CD4^+$ T cells, respectively (direct MHC allorecognition). Host DCs from the recipient may also migrate into the graft, pick up graft alloantigens, and transport these back to the draining lymph nodes, where they are displayed (the indirect pathway). The connection between lymphatic vessels in allografts and the recipient's lymph nodes is surgically disrupted during the process of transplantation, and it is likely reestablished by growth of new lymphatic channels in response to inflammatory stimuli produced during grafting. Naive $CD4^+$ and $CD8^+$ lymphocytes that normally traffic through the lymph node encounter these alloantigens and are induced to proliferate and differentiate into effector helper T cells and cytotoxic T lymphocytes (CTLs). This process is sometimes called sensitization to alloantigens. The effector cells migrate back into the graft and mediate rejection, by mechanisms that are discussed later.

As mentioned earlier, many of the T cells that respond to the allogeneic MHC antigens in a new graft are cross-reactive memory T cells previously generated against environmental antigens before transplantation. Unlike naive T cells, memory T cells may not need to see antigens presented by DCs in lymph nodes to be activated, and they may migrate directly into grafts, where they can be activated by APCs or tissue cells displaying alloantigen.

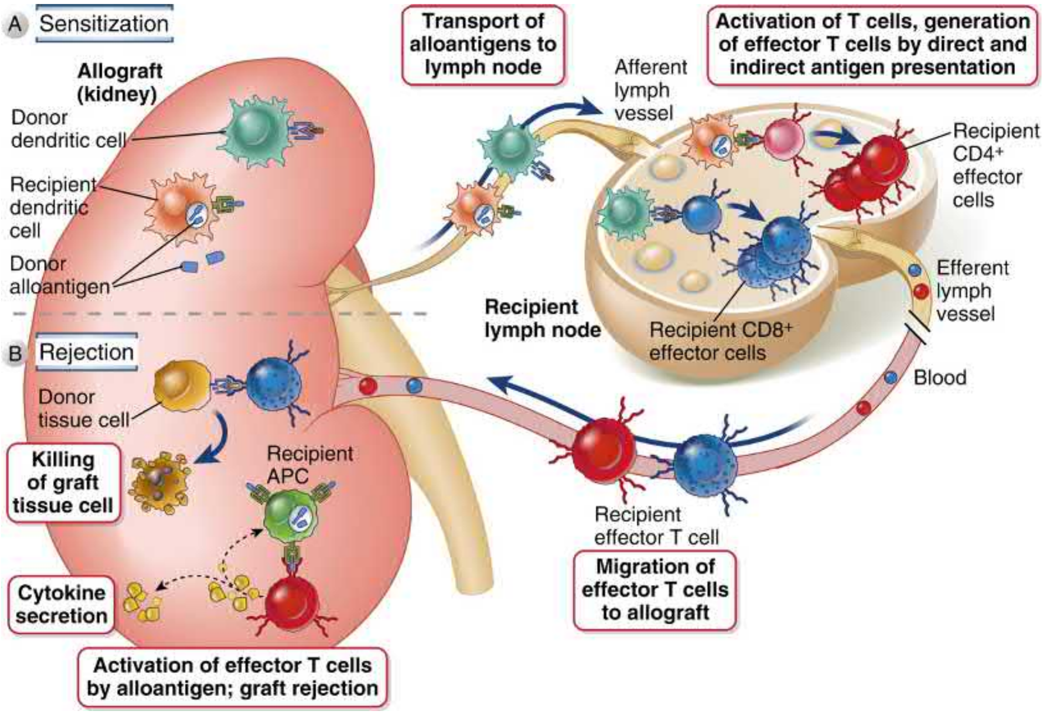

A Sensitization
Allograft (kidney)
Donor dendritic cell
Recipient dendritic cell
Donor alloantigen
Transport of alloantigens to lymph node
Afferent lymph vessel
Activation of T cells, generation of effector T cells by direct and indirect antigen presentation
Recipient CD4+ effector cells
Efferent lymph vessel
Recipient lymph node
Recipient CD8+ effector cells
Blood
B Rejection
Donor tissue cell
Killing of graft tissue cell
Recipient APC
Cytokine secretion
Activation of effector T cells by alloantigen; graft rejection
Recipient effector T cell
Migration of effector T cells to allograft

FIGURE 17.6 Activation of alloreactive T cells. **A,** In the case of direct allorecognition, donor dendritic cells in the allograft migrate to secondary lymphoid tissues, where they directly present allogeneic major histocompatibility complex *(MHC)* molecules to host T cells. Only CD8$^+$ T cells recognizing donor class I MHC is shown, but CD4$^+$ T cells can also directly recognize donor class II MHC. In the case of indirect allorecognition, recipient dendritic cells that have entered the allograft transport donor MHC proteins to secondary lymphoid tissues and present peptides derived from these MHC proteins to alloreactive host T cells. This is shown for CD4$^+$ T cells, and indirect recognition of allogeneic MHC by CD8$^+$ T cells is likely less important. After both indirect and direct allorecognition, the T cells become activated and differentiate into effector CD4$^+$ helper T cells and CD8$^+$ cytotoxic T lymphocytes (CTLs). **B,** The alloreactive effector T cells

> migrate into the allograft, become reactivated by alloantigen, and mediate damage. In the graft, direct recognition of allogeneic class I MHC by $CD8^+$ CTLs is required for killing of graft parenchymal cells, because these cells express only allogeneic MHC. In contrast, $CD4^+$ helper T cells that can directly or indirectly recognize allogeneic class II MHC can be activated by donor or host antigen-presenting cells *(APCs),* respectively, and both can promote inflammation that damages the graft.

The response of alloreactive T cells to foreign MHC molecules can be analyzed in vitro by the **mixed lymphocyte reaction** (MLR), in which lymphocytes from two genetically distinct individuals are mixed together in cell culture. The T cells from one individual become activated by recognition of allogeneic MHC molecules on the cells of the other. The MLR was used clinically in the past as a predictive test of T cell–mediated graft rejection and as an in vitro model to study the mechanisms of alloreactivity, but now it is mainly of historical significance.

Role of Costimulation in T Cell Responses to Alloantigens

In addition to recognition of alloantigen, costimulation of T cells primarily by B7 molecules on APCs is important for activating alloreactive T cells. Costimulation is likely most important to activate naive alloreactive T cells, but even alloreactive memory T cell responses can be enhanced by costimulation. Rejection of allografts and stimulation of alloreactive T cells in an MLR can be inhibited by agents that block B7 molecules. Allografts survive for longer periods when they are transplanted into knockout mice lacking B7-1 (CD80) and B7-2 (CD86) compared with transplants into normal recipients. As we will discuss later, blocking B7 costimulators is a therapeutic strategy to inhibit graft rejection in humans as well.

The requirement for costimulation leads to the interesting question of why these costimulators are expressed by graft APCs in the absence of infection, which we have previously discussed as the physiologic stimulus for the expression of costimulators (see Chapter 9). One possibility is that the innate immune response to ischemic damage of some cells in the graft, discussed later, results in increased expression of costimulators on APCs.

Effector Functions of Alloreactive T Cells

Alloreactive CD4$^+$ and CD8$^+$ T cells that are activated by graft alloantigens cause rejection by distinct mechanisms (see Fig. 17.6) **.** The CD4$^+$ helper T cells differentiate into cytokine-producing effector cells that damage grafts by cytokine-mediated inflammation, similar to a delayed-type hypersensitivity (DTH) reaction (see Chapters 10 and 19). CD8$^+$ T cells differentiate into CTLs, which kill graft cells.

Only CTLs that are generated by direct allorecognition can kill graft cells, whereas both CTLs and helper T cells generated by either direct or indirect alloantigen recognition can cause cytokine-mediated damage to grafts. CD8$^+$ CTLs that are generated by direct allorecognition of donor MHC molecules on donor APCs can recognize the same MHC molecules on parenchymal cells in the graft and kill those cells. These T cells can also secrete cytokines that cause damaging inflammation. In contrast, any CD8$^+$ CTLs that are generated in response to indirect recognition of allogeneic MHC are restricted to recognition of peptides from these allogeneic MHC molecules bound to recipient (self) MHC molecules, and therefore the T cells will not be able to kill the foreign graft cells because the graft cells do not express recipient MHC molecules. When CD4$^+$ effector T cells are generated by direct or indirect recognition of allogeneic MHC, the principal mechanism of rejection is inflammation caused by the cytokines produced by the effector T cells. CD8$^+$ T cells that may be activated by the indirect pathway may also contribute to rejection by producing inflammatory cytokines. Presumably, effector cells activated by the indirect pathway infiltrate the graft and recognize peptides from graft MHC molecules being displayed by host APCs that have also entered the graft.

Activation of Alloreactive B Cells and Production and Functions of Alloantibodies

Antibodies against graft antigens, called donor-specific antibodies, also contribute to rejection. High-affinity alloantibodies are mostly produced by helper T cell–dependent activation of alloreactive B cells, much like antibodies against other protein antigens (see Chapter 12). The antigens most frequently recognized by alloantibodies are donor MHC molecules, including both class I and class II MHC proteins. The likely sequence of events leading to the generation of these alloantibody-producing cells is that naive B lymphocytes recognize the allogenic MHC molecules, internalize and process these proteins, and present peptides derived from them to helper T cells that were previously activated by the same peptides presented by DCs (see Fig. 17.4). Thus, activation of alloreactive B cells is an example of indirect presentation of alloantigens. In addition, donor-specific antibodies against non-HLA alloantigens also contribute to rejection.

The alloreactive antibodies produced in graft recipients engage the same effector mechanisms that antibodies use to combat infections, including complement activation, and Fc receptor–mediated binding and activation of neutrophils, macrophages, and NK cells. Because MHC antigens are expressed on endothelial cells, much of the alloantibody-mediated damage is targeted at the graft vasculature, as discussed later.

Innate Immune Responses to Allografts

In addition to the adaptive immune responses specific for alloantigenic differences between donor and host, innate immunity plays a role in the outcome of transplantation. The interruption of blood supply to tissues and organs during the time between removal from a donor and placement in a host usually causes some ischemic damage. This can result in expression of damage-associated molecular patterns (DAMPs) in the graft (see Chapter 4), which simulate innate responses mediated by both innate cells within the graft and the recipient's innate immune system. These innate responses can directly cause graft injury, but they are also thought to enhance adaptive responses by increasing migration of alloreactive circulating memory T cells into the graft and by activating APCs, as is the case in immune responses to microbes (see Chapter 6). APC activation increases the expression of costimulators and production of cytokines and thus the priming of naive alloreactive T lymphocytes. In addition, host NK cells can respond to the absence of self MHC molecules on donor graft cells (see Chapter 4) and therefore contribute to graft rejection.

Patterns and Mechanisms of Allograft Rejection

Thus far, we have described the molecular basis of alloantigen recognition and the cells involved in the recognition of and responses to allografts. We now turn to a consideration of the effector mechanisms responsible for the immunologic rejection of allografts. In different experimental models and in clinical transplantation, alloreactive CD4$^+$ and CD8$^+$ T cells and alloantibodies all have been shown to be capable of mediating allograft rejection. These different immune effectors cause graft rejection by different mechanisms, and all three effectors may contribute to rejection concurrently.

For historical reasons, graft rejection is classified on the basis of the time course of rejection after transplantation and histopathologic features rather than by immune effector mechanisms. Based on the experience of renal transplantation, the histopathologic patterns are called hyperacute, acute, and chronic. It is helpful to consider rejection in the context of these patterns because the immune mechanism of rejection correlates well with the pattern. Our discussion of these types of rejection will emphasize the underlying mechanisms rather than the pathologic or clinical features.

Hyperacute Rejection

Hyperacute rejection is characterized by thrombotic occlusion of the graft vasculature that begins within minutes to hours after host blood vessels are anastomosed to graft vessels and is mediated by preexisting antibodies in the host circulation that bind to donor endothelial antigens (Fig. 17.7A) **.** Binding of antibody to endothelium activates complement, and antibody and complement products together induce a number of changes in the graft endothelium that promote intravascular thrombosis. Complement activation leads to endothelial cell injury and exposure of subendothelial basement membrane proteins that activate platelets. The endothelial cells are stimulated to secrete high-molecular-weight forms of von Willebrand factor, which causes platelet adhesion and aggregation. Both endothelial cells and platelets undergo membrane vesiculation, leading to shedding of lipid particles that promote coagulation. Endothelial cells lose the cell surface heparan sulfate proteoglycans that normally interact with antithrombin III to inhibit coagulation. These processes contribute to thrombosis and vascular occlusion (Fig. 17.7B), and the grafted organ suffers irreversible ischemic necrosis.

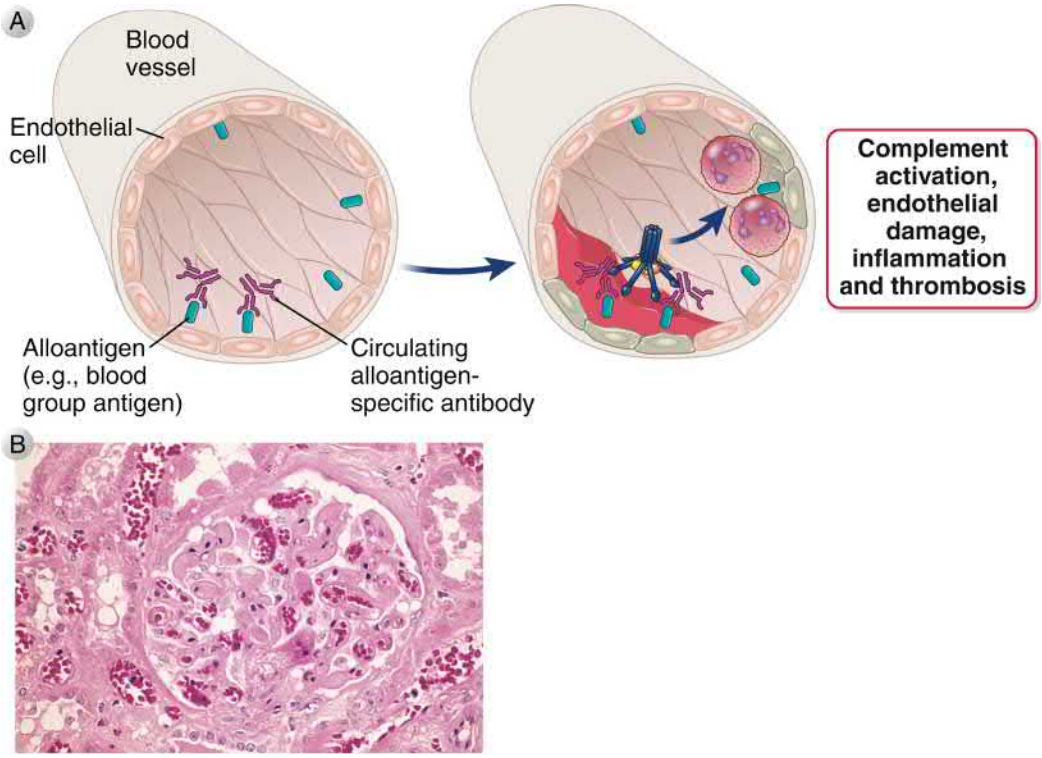

A
Blood vessel
Endothelial cell
Alloantigen (e.g., blood group antigen)
Circulating alloantigen-specific antibody
Complement activation, endothelial damage, inflammation and thrombosis
B

FIGURE 17.7 Hyperacute rejection. **A,** In hyperacute rejection, preformed antibodies reactive with vascular endothelium activate complement and trigger rapid intravascular thrombosis and necrosis of the vessel wall. **B,** Hyperacute rejection involving a glomerulus in a kidney allograft. Typical features include endothelial damage, thrombi, and leukocytic infiltration.

B, Courtesy Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.

In the early days of transplantation, hyperacute rejection was often mediated by preexisting IgM alloantibodies specific for the carbohydrate ABO blood group antigens that are expressed on red blood cells and endothelial cells. These natural antibodies are present in most individuals (discussed later). Hyperacute rejection by anti-ABO antibodies is extremely rare now because all donor and recipient pairs are selected so that they have compatible ABO types. Hyperacute rejection caused by natural antibodies, specific for a variety of antigens that differ among species, is a major barrier to xenotransplantation and limits the use of animal organs for human transplantation.

Currently, the rare instances of hyperacute rejection of allografts that do occur are mediated by IgG antibodies directed against protein alloantigens, such as donor MHC molecules, or against less defined alloantigens expressed on vascular endothelial cells. Such antibodies generally arise as a result of previous exposure to alloantigens through blood transfusion, previous transplantation, or multiple pregnancies. If the level of these alloreactive antibodies is low, hyperacute rejection may develop slowly, over several days, but the onset is still earlier than that typical for acute rejection. As we will discuss later, patients in need of allografts are routinely screened before grafting for the presence of antibodies that bind to blood cells of a potential organ donor to avoid the use of organs that will likely suffer hyperacute rejection.

In unusual cases in which grafts have to be done between ABO-incompatible donors and recipients, graft survival may be improved by rigorous depletion of antibodies and B cells. Sometimes, if the graft is not rapidly rejected, it survives even in the presence of anti-graft antibody. One possible mechanism of this resistance to hyperacute rejection is increased expression of complement regulatory proteins on graft endothelial cells, a beneficial adaptation of the tissue called accommodation.

Acute Rejection

Acute rejection is a process of injury to the graft parenchyma and blood vessels mediated by alloreactive T cells and antibodies. Before modern immunosuppression, acute rejection would often begin several days to a few weeks after transplantation. The time of onset of acute rejection reflects the time needed to generate alloreactive effector T cells and antibodies in response to the graft. In current clinical practice, episodes of acute rejection may occur at much later times, even years after transplantation, if immunosuppression is reduced for any number of reasons. Although the patterns of acute rejection are divided into acute T cell–mediated (cellular) rejection and acute antibody-mediated (humoral) rejection, both often coexist in an organ undergoing acute rejection.

Acute Cellular Rejection

The principal mechanisms of acute cellular rejection are CTL-mediated killing of graft parenchymal cells and endothelial cells and inflammation caused by cytokines produced by helper T cells (Fig. 17.8A) **.** On histologic examination of kidney allografts, where this type of rejection is best characterized, there are infiltrates of lymphocytes and macrophages. In kidney allografts, the infiltrates may involve the tubules (called tubulitis; Fig. 17.8B), with associated tubular necrosis, and blood vessels (called endotheliitis; Fig. 17.8C), with necrosis of the walls of capillaries and small arteries. The cellular infiltrates present in grafts undergoing acute cellular rejection include both $CD4^+$ helper T cells and $CD8^+$ CTLs specific for graft alloantigens, and both types of T cells may cause parenchymal cell and endothelial injury. The helper T cells are mainly Th1 cells that produce interferon-γ (IFN-γ) and tumor necrosis factor (TNF). They cause macrophage and endothelial activation and inflammatory damage to the organ. Experimentally, adoptive transfer of alloreactive $CD4^+$ helper T cells or $CD8^+$ CTLs can cause acute cellular graft rejection in recipient mice.

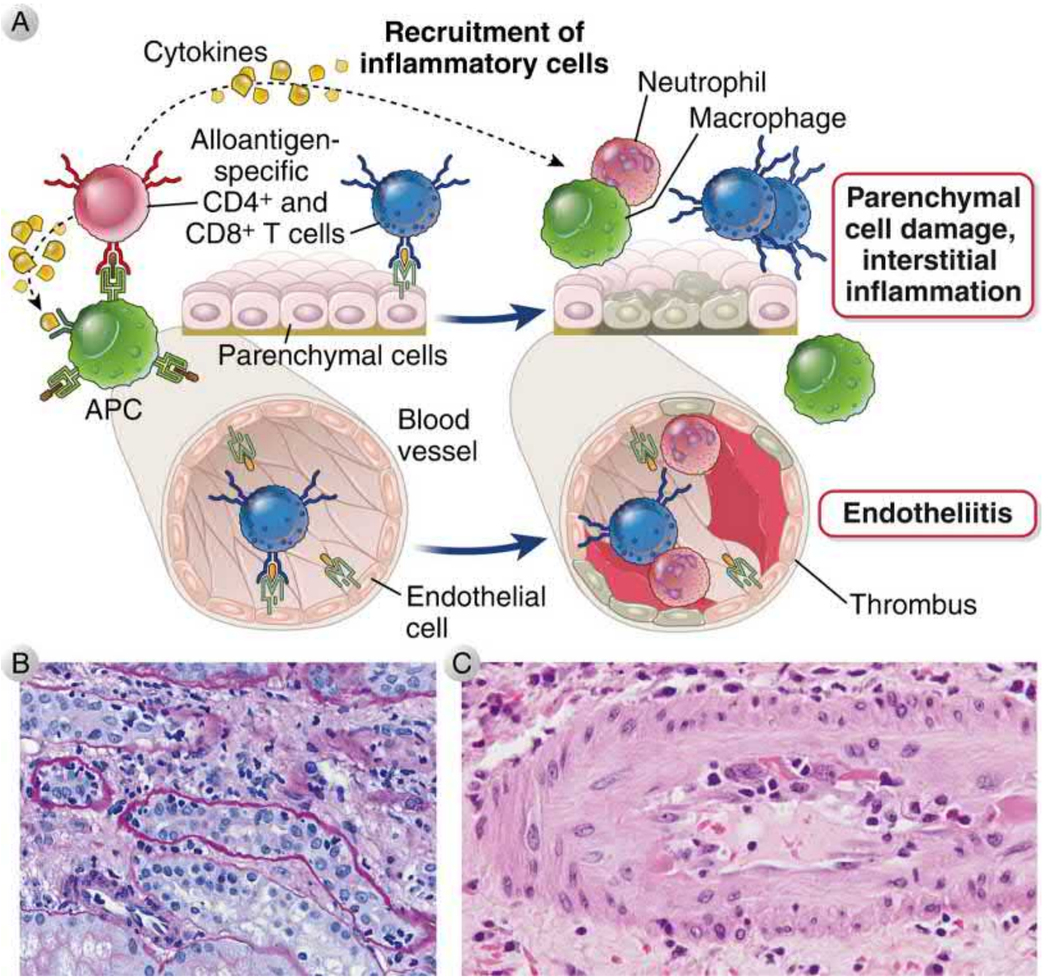

A
Cytokines
Recruitment of inflammatory cells
Neutrophil
Macrophage
Alloantigen-specific CD4+ and CD8+ T cells
Parenchymal cell damage, interstitial inflammation
Parenchymal cells
APC
Blood vessel
Endotheliitis
Endothelial cell
Thrombus
B
C

FIGURE 17.8 Acute cellular rejection. **A,** In acute cellular rejection, CD4$^+$ and CD8$^+$ T lymphocytes reactive with alloantigens on endothelial cells in blood vessels and parenchymal cells mediate damage to these cell types. **B,** Acute cellular rejection of a kidney with inflammatory cells in the connective tissue around the tubules and between epithelial cells of the tubules. **C,** Inflammation of the endothelial layer of a blood vessel (endotheliitis) in acute cellular rejection, with inflammatory cells damaging endothelium.

B, Courtesy Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts. *C,* Courtesy Dr. Zoltan Laszik, Department of Pathology, University of California, San Francisco, California.

Acute Antibody-Mediated (Humoral) Rejection

Alloantibodies cause acute rejection by binding to alloantigens, mainly HLA molecules, on vascular endothelial cells, leading to endothelial injury and intravascular thrombosis that result in graft destruction (Fig. 17.9A) **.** The binding of the alloantibodies to the endothelial cell surface triggers local complement activation, which causes lysis of the cells, recruitment and activation of neutrophils, and thrombus formation. Alloantibodies may also engage Fc receptors on neutrophils and NK cells, which then kill the endothelial cells. In addition, alloantibody binding to the endothelial surface may directly alter endothelial function by inducing intracellular signals that enhance surface expression of proinflammatory and procoagulant molecules.

The histologic hallmarks of acute antibody-mediated rejection of renal allografts are acute inflammation of glomeruli and peritubular capillaries with focal capillary thrombosis (Fig. 17.9B). Immunohistochemical identification of the C4d complement fragment in capillaries of renal allografts is used clinically as an indicator of activation of the classical complement pathway and humoral rejection (Fig. 17.9C).

Chronic Rejection

As therapy for acute rejection has improved, the major cause of the failure of vascularized organ allografts has become chronic rejection. Since 1990, 1-year survival of kidney allografts has been better than 90%, but the 10-year survival has remained approximately 60% despite advances in immunosuppressive therapy. Chronic rejection develops insidiously during months or years and may or may not be preceded by clinically recognized episodes of acute rejection. Chronic rejection of different transplanted organs is associated with distinct pathologic changes. In the kidney and heart, chronic rejection results in vascular occlusion and interstitial fibrosis. Lung transplants undergoing chronic rejection show thickened small airways (called bronchiolitis obliterans), and liver transplants show fibrotic and nonfunctional bile ducts.

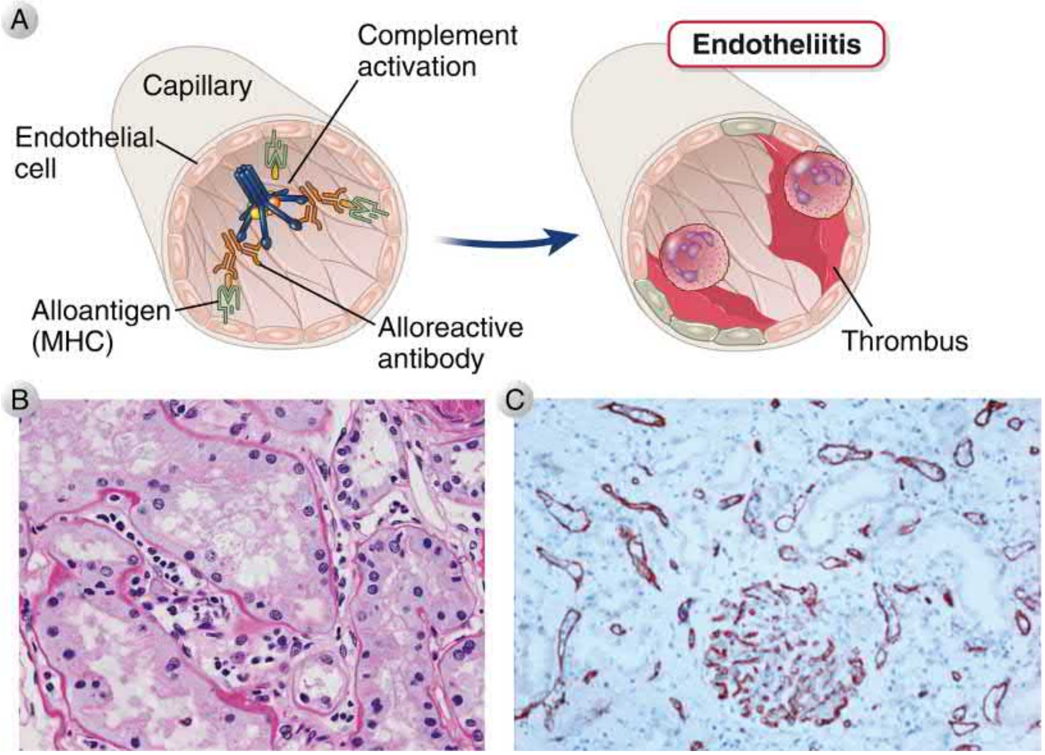

A
Complement activation
Capillary
Endothelial cell
Alloantigen (MHC)
Alloreactive antibody
Endotheliitis
Thrombus
B
C

FIGURE 17.9 Acute antibody-mediated rejection. **A,** Alloreactive antibodies formed after engraftment may contribute to parenchymal and vascular injury. **B,** Acute antibody-mediated rejection of a kidney allograft with inflammatory cells in peritubular capillaries. **C,** Complement C4d deposition in capillaries in acute antibody-mediated rejection, revealed by immunohistochemistry as brown staining. *MHC,* Major histocompatibility complex.

B and *C,* Courtesy Dr. Zoltan Laszik, Department of Pathology, University of California, San Francisco, California.

A dominant lesion of chronic rejection in vascularized grafts is arterial occlusion as a result of the proliferation of intimal smooth muscle cells, and the grafts eventually fail mainly because of the

resulting ischemic damage (Fig. 17.10) **.** The arterial changes are called graft vasculopathy or accelerated graft arteriosclerosis (Fig. 17.10B). Graft vasculopathy is frequently seen in failed cardiac and renal allografts and can develop in any vascularized organ transplant within 6 months to a year after transplantation. The likely mechanisms underlying the occlusive vascular lesions of chronic rejection are activation of alloreactive T cells and secretion of IFN-γ and other cytokines that stimulate proliferation of vascular smooth muscle cells. As the arterial lesions of graft arteriosclerosis progress, blood flow to the graft parenchyma is compromised, and the parenchyma is slowly replaced by nonfunctioning fibrous tissue (Fig. 17.10C). The interstitial fibrosis seen in chronic rejection also may be a repair response to parenchymal cell damage caused by repeated bouts of acute antibody-mediated or cellular rejection, perioperative ischemia, toxic effects of immunosuppressive drugs, and even chronic viral infections. Chronic rejection leads to congestive heart failure or arrhythmias in cardiac transplant patients or loss of glomerular and tubular function and renal failure in kidney transplant patients.

Prevention and Treatment of Allograft Rejection

If the recipient of an allograft has a fully functional immune system, transplantation almost invariably results in some form of rejection. The strategies used in clinical practice and in experimental models to avoid or delay rejection are general immunosuppression and minimizing the strength of the specific allogeneic reaction. An important goal of transplantation research is to find ways of inducing donor-specific tolerance, which would allow grafts to survive without nonspecific immunosuppression.

Methods to Reduce the Immunogenicity of Allografts

Solid organs used in transplantation come from both living and deceased donors, and graft survival after transplantation varies depending on the source. The greatest barrier to transplantation as a therapeutic option for organ failure is availability of organs. Currently in the United States, there are approximately 110,000 people in need of a life-saving organ transplant, but there have been only approximately 20,000 donors per year. Living donors can donate one kidney, a lobe of a lung, and parts of the liver, pancreas, or intestine, because they can remain healthy after these types of donations. Living donors may be genetically related to the recipient, including siblings, parents, children (over 18 years of age), aunts, uncles, cousins, nieces, and nephews. Other living donors may be unrelated. As we have discussed, immunologic graft rejection is targeted at allogeneic proteins encoded by polymorphic alleles in the recipient not shared by the donor. Related donors will share more alleles of polymorphic genes, including MHC genes, than unrelated donors, and this will reduce the incidence and severity of rejection episodes (as discussed later). For example, because MHC genes are inherited as linked haplotypes, there is a 25% chance that two siblings will have identical MHC genes, whereas the chance of an unrelated donor and recipient having identical MHC genes is extremely low.

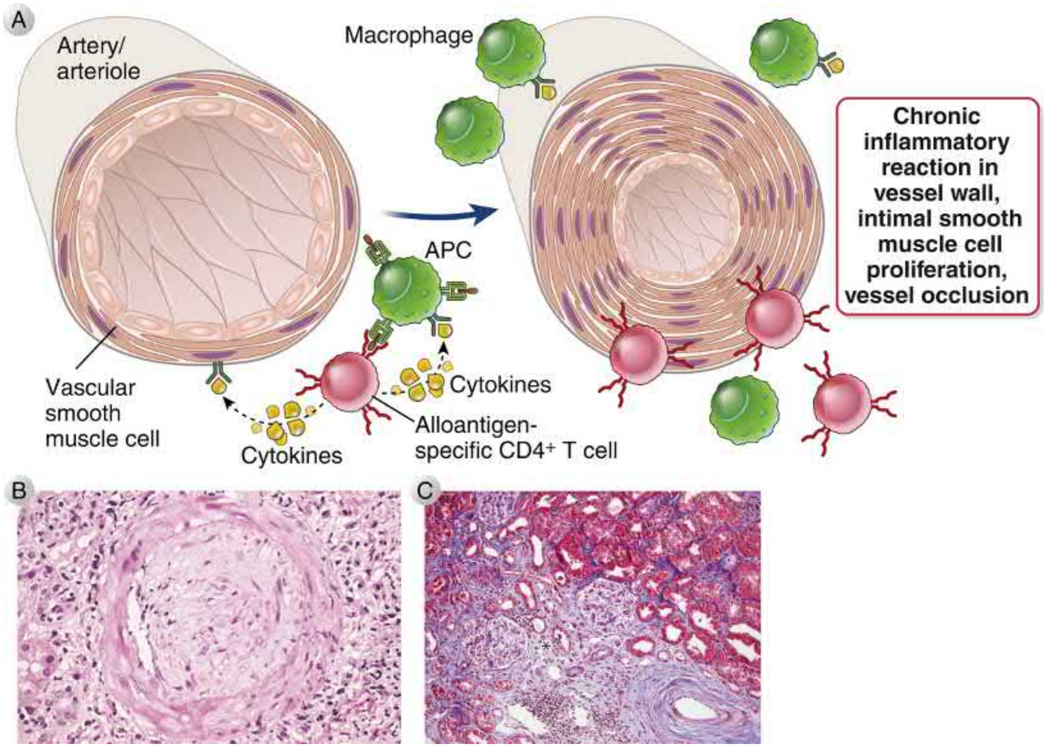

A
Artery/ arteriole
Macrophage
Chronic inflammatory reaction in vessel wall, intimal smooth muscle cell proliferation, vessel occlusion
APC
Vascular smooth muscle cell
Cytokines
Cytokines
Alloantigen-specific CD4+ T cell
B
C

FIGURE 17.10 Chronic rejection. **A,** In chronic rejection with graft arteriosclerosis, injury to the vessel wall leads to intimal smooth muscle cell proliferation and luminal occlusion. This lesion may be caused by a chronic inflammatory reaction to alloantigens in the vessel wall. **B,** Chronic rejection in a kidney allograft with graft arteriosclerosis. The vascular lumen is replaced by an accumulation of smooth muscle cells and connective tissue in the vessel intima. **C,** Fibrosis and loss of tubules in a kidney with chronic rejection *(lower left)* adjacent to relatively normal kidney *(upper right)*. The *blue area* shows fibrosis, and an artery with graft arteriosclerosis is present *(bottom right)*.

B, Courtesy Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts. *C,* Courtesy Dr. Zoltan Laszik, Department of Pathology, University of California, San Francisco, California.

Deceased donors, called cadaveric donors, are sources of any transplantable organ and the only source of organs that could not be removed from a living donor, such as hearts. Most deceased donors are brain dead, with complete and irreversible loss of all higher brain function, but whose other organs can be kept alive in the body by cardiorespiratory life support, until just before organ harvest. Less frequently, organs are retrieved from people after very recent but irreversible cessation of circulation and respiration, such as after trauma. The survival of grafts from deceased donors is on average lower than from either related or unrelated living donors because there is more ischemic damage to organs removed after death of the donor. Furthermore, most deceased donors are unrelated to the recipients, and grafts from unrelated donors usually express more antigens that differ from those of the recipient and can simulate stronger rejection responses than those from living donors.

In human transplantation, the major strategy to reduce graft immunogenicity has been to minimize alloantigenic differences between the donor and recipient. Several clinical laboratory tests are routinely performed to reduce the risk for immunologic rejection of allografts. These include ABO blood typing; the determination of HLA alleles expressed on donor and recipient cells, called tissue typing; the detection of preformed antibodies in the recipient that recognize HLA and other antigens representative of the donor population; and the detection of preformed antibodies in the recipient that bind to antigens of an identified donor's cells, called cross-matching. Not all of these tests are done in all types of transplantation. We will next summarize each of these tests and discuss their significance.

To avoid hyperacute rejection, the ABO blood group antigens of the graft donor are selected to be compatible with the recipient. This test is uniformly used in organ transplantation because grafts will typically not survive if there are ABO incompatibilities between the donor and recipient. Natural IgM antibodies specific for allogeneic ABO blood group antigens will cause hyperacute rejection. Blood typing is performed by mixing a patient's red blood cells with standardized sera containing anti-A or anti-B antibodies. If the patient expresses either blood group antigen, the serum specific for that antigen will agglutinate the red blood cells. The biology of the ABO blood group system is discussed later in this chapter in the context of blood transfusion.

In kidney transplantation, the more MHC alleles that are matched between the donor and recipient, the better the graft survival (Fig. 17.11) **.** HLA matching had a more profound influence on graft survival before modern immunosuppressive drugs were routinely used, but current data still show significantly greater survival of grafts when donor and recipient have fewer HLA allele mismatches. Past clinical experience with older typing methods showed that of all class I and class II MHC loci, matching at HLA-A, HLA-B, and HLA-DR is most important for predicting survival of kidney allografts. (HLA-C is not as polymorphic as HLA-A or HLA-B, and HLA-DR and HLA-DQ are in linkage disequilibrium, so matching at the DR locus often also matches at the DQ locus.) Although current typing protocols in many centers include HLA-C, HLA-DQ, and HLA-DP loci, most of the available data in predicting graft outcome refer only to HLA-A, HLA-B, and HLA-DR mismatches. Because two codominantly expressed alleles are inherited for each of these HLA genes, it is possible to have zero to six HLA mismatches of these three loci between the donor and recipient. Zero-antigen mismatches predict the best survival of living related donor grafts, and grafts with one-antigen mismatches are similar. The survival of grafts with two to six HLA mismatches is significantly worse than that of grafts with zero- and one-antigen mismatches. Mismatching of two or more HLA genes has an even greater impact on nonliving (unrelated) donor renal allografts. Therefore, attempts are made to reduce the number of differences in HLA alleles expressed on donor and recipient cells, which will have a modest effect in reducing the chance of rejection.

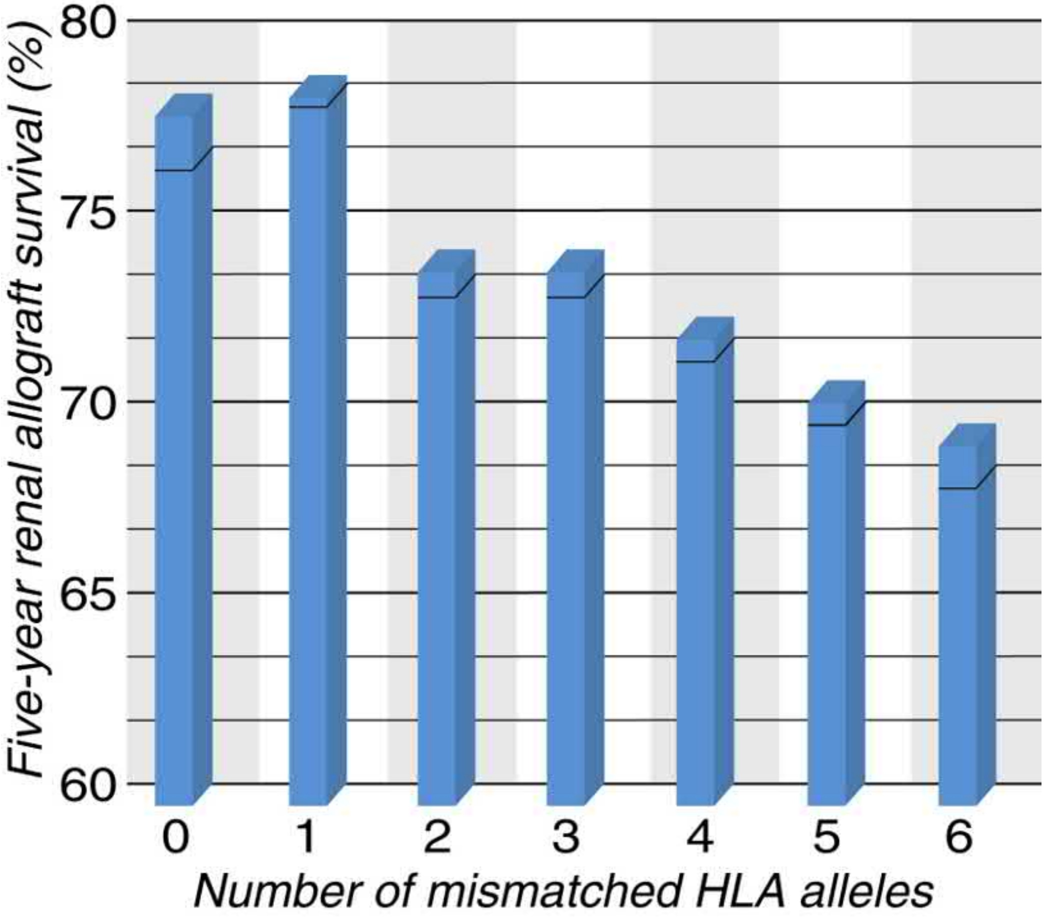


FIGURE 17.11 Influence of major histocompatibility complex matching on graft survival.Matching of major histocompatibility complex *(MHC)* alleles between the donor and recipient significantly improves renal allograft survival. The data shown are for deceased donor (cadaver) grafts. Human leukocyte antigen *(HLA)* matching has less of an impact on survival of renal allografts from live donors, and some *MHC* alleles are more important than others in determining outcome. Data from Scientific Registry of Transplant Recipients. SRTR annual report, 2012. Available at http://www.srtr.org/. Accessed July 2013.

HLA matching in renal transplantation is possible because donor kidneys can be stored for some time before being transplanted, and patients needing a kidney allograft can be maintained on dialysis until a well-matched organ is available. In the case of heart and liver transplantation, organ preservation is more difficult, and potential recipients are often in critical condition. For these reasons, HLA typing is not considered in pairing of potential donors and recipients, and the choice of donor and recipient is based on ABO blood group matching, other measures of immunologic compatibility described later, and anatomic compatibility. The paucity of heart donors, the emergent need for transplantation, and the success of immunosuppression override any benefit of reducing HLA mismatches between donor and recipient. As we will discuss later, in HSC transplantation, HLA matching is essential to reduce the risk for graft-versus-host disease (GVHD).

Most HLA haplotype determinations are now performed by polymerase chain reaction (PCR), replacing older serologic methods. MHC genes can be amplified by PCR with use of primers that bind to nonpolymorphic sequences within the 5′ and 3′ ends of exons encoding the polymorphic regions of class I and class II MHC molecules. The amplified segment of DNA can then be sequenced. Thus, the actual nucleotide sequence and therefore the predicted amino acid sequence can be directly determined for the MHC alleles of any cell, providing precise molecular tissue typing. On the basis of these DNA sequencing efforts, the nomenclature of HLA alleles has changed to reflect the identification of many alleles not distinguished by previous serologic methods. Each allele defined by sequence has at least a four-digit number, but some alleles require six or eight digits for precise definition. The first two digits usually correspond to the older serologically defined allotype, and the third and fourth digits indicate the subtypes. Alleles with differences in the first four digits encode proteins with different amino acids. For example, HLA-DRB1∗1301 is the sequence-defined 01 allele of the serologically defined HLA-DR13 family of genes encoding the HLA-DR β1 protein.

Patients in need of allografts are also tested for the presence of preformed antibodies against donor MHC molecules or other cell

surface antigens. Two types of tests are done to detect these antibodies. In the panel reactive antibody (PRA) test, patients waiting for organ transplants are screened for the presence of preformed antibodies reactive with allogeneic HLA molecules prevalent in the population. The presence of these antibodies, which may be produced as a result of previous pregnancies, transfusions, or transplantation, increases risk for hyperacute or acute vascular rejection. Small amounts of the patient's serum are mixed with multiple fluorescently labeled beads coated with defined MHC molecules, representative of the MHC alleles that may be present in an organ donor population. Each MHC molecule is attached to a bead with a differently colored fluorescent label. Binding of the patient's antibodies to beads is determined by flow cytometry. The results are reported as the percentage of the MHC allele panel with which the patient's serum reacts. The PRA is determined on multiple occasions while a patient waits for an organ allograft. This is because the PRA can vary, as each panel is chosen at random and the patient's serum antibody titers may change over time.

If a potential donor is identified, the cross-matching test will determine if the patient has antibodies that react specifically with that donor's cells. The test is performed by mixing the recipient's serum with the donor's blood lymphocytes (a convenient source of cells, some of which express both class I and class II MHC proteins). Complement-mediated cytotoxicity tests or flow cytometric assays can then be used to determine if antibodies in the recipient serum have bound to the donor cells. For example, complement is added to the mixture of cells and serum, and if preformed antibodies, usually against donor MHC molecules, are present in the recipient's serum, the donor cells are lysed. This would be a positive cross-match, which indicates that the donor is not suitable for that recipient.

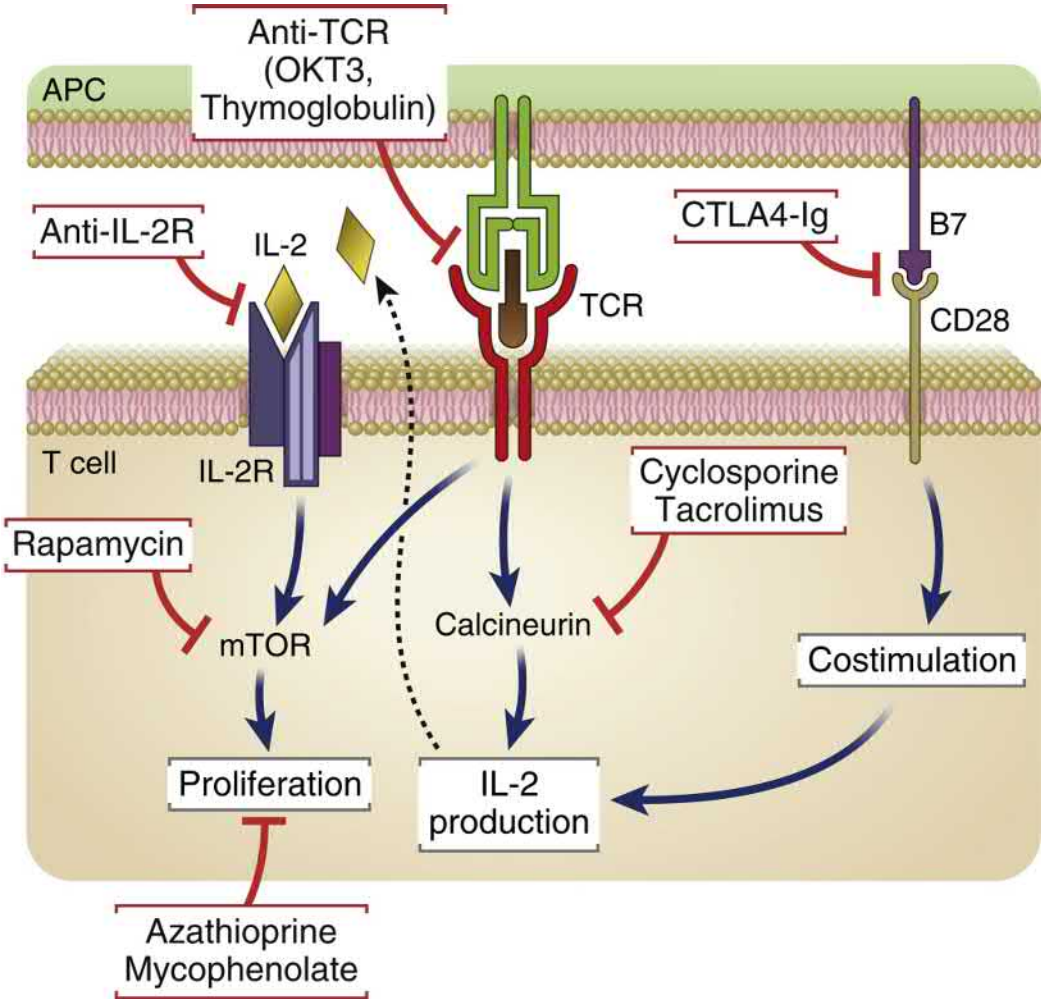

Anti-TCR (OKT3, Thymoglobulin)
APC
Anti-IL-2R
IL-2
CTLA4-Ig
B7
TCR
CD28
T cell
IL-2R
Cyclosporine Tacrolimus
Rapamycin
mTOR
Calcineurin
Costimulation
Proliferation
IL-2 production
Azathioprine Mycophenolate

FIGURE 17.12 Mechanisms of action of immunosuppressive drugs.Each major category of drugs used to prevent or to treat allograft rejection is shown along with the molecular targets of the drugs. *APC,* Antigen-presenting cell; *CTL,* cytotoxic T lymphocyte; *IL,* interleukin; *mTOR,* mechanistic target of rapamycin; *TCR,* T cell receptor.

Immunosuppression to Prevent or to Treat Allograft Rejection

Immunosuppressive drugs that inhibit or kill T lymphocytes are the principal agents used to treat or prevent graft rejection. Several drugs are commonly used (Fig. 17.12).

Inhibitors of T Cell Signaling Pathways

The calcineurin inhibitors cyclosporine and tacrolimus (FK506) inhibit transcription of certain genes in T cells, most notably genes encoding cytokines such as IL-2. Cyclosporine is a fungal peptide that binds with high affinity to a ubiquitous cellular protein called cyclophilin. The complex of cyclosporine and cyclophilin binds to and inhibits the enzymatic activity of calcineurin, a calcium/calmodulin-activated serine/threonine phosphatase (see Chapter 7). Because calcineurin is required to activate the transcription factor nuclear factor of activated T cells (NFAT), cyclosporine inhibits NFAT activation and the transcription of IL-2 and other cytokine genes. The net result is that cyclosporine blocks the IL-2–dependent proliferation and differentiation of T cells. Tacrolimus is a macrolide made by a bacterium that functions like cyclosporine. Tacrolimus binds to FK506 binding protein (FKBP), and the complex shares with the cyclosporine-cyclophilin complex the ability to bind calcineurin and inhibit its activity.

The introduction of cyclosporine into clinical practice ushered in the modern era of transplantation. Before the use of cyclosporine, the majority of transplanted hearts and livers were rejected. Now as a result of the use of cyclosporine, tacrolimus, and other more recently introduced drugs, the majority of these allografts survive for more than 5 years (Fig. 17.13). Nevertheless, calcineurin inhibitors have limitations. For example, at doses needed for optimal immunosuppression, cyclosporine causes kidney damage, and some rejection episodes are refractory to cyclosporine treatment. Tacrolimus was initially used for liver transplant recipients, but it has now largely replaced cyclosporine for all organ transplants because of better efficacy and safety.

The immunosuppressive drug rapamycin (sirolimus) inhibits growth factor–mediated T cell proliferation. Like tacrolimus, rapamycin binds to FKBP, but the rapamycin-FKBP complex does not inhibit calcineurin. Instead, this complex binds to and inhibits a cellular enzyme called mTOR (mechanistic target of rapamycin), which is a serine/threonine protein kinase required for translation of proteins that promote cell survival and proliferation. mTOR is negatively regulated by a protein complex called tuberous sclerosis complex 1 (TSC1)-TSC2 complex. Phosphatidylinositol 3-kinase (PI3K)-AKT signaling results in phosphorylation of TSC2 and release of mTOR inhibition. Several growth factor receptor signaling pathways, including the IL-2 receptor pathway in T cells, as well as TCR and CD28 signals, activate mTOR through PI3K-AKT, leading to translation of proteins needed for cell cycle progression. Thus, by inhibiting mTOR function, rapamycin blocks T cell proliferation. Combinations of calcineurin inhibitors (which block IL-2 synthesis) and rapamycin (which blocks IL-2–driven proliferation) potently inhibit T cell responses. Interestingly, rapamycin inhibits the generation of effector T cells but does not impair the survival and functions of regulatory T cells (Tregs) as much, which may promote immune suppression of allograft rejection. mTOR is involved in DC functions, and therefore, rapamycin may suppress T cell responses by its effects on DCs as well. mTOR is also involved in B cell proliferation and antibody responses, and therefore rapamycin also may be effective in preventing or treating antibody-mediated rejection.

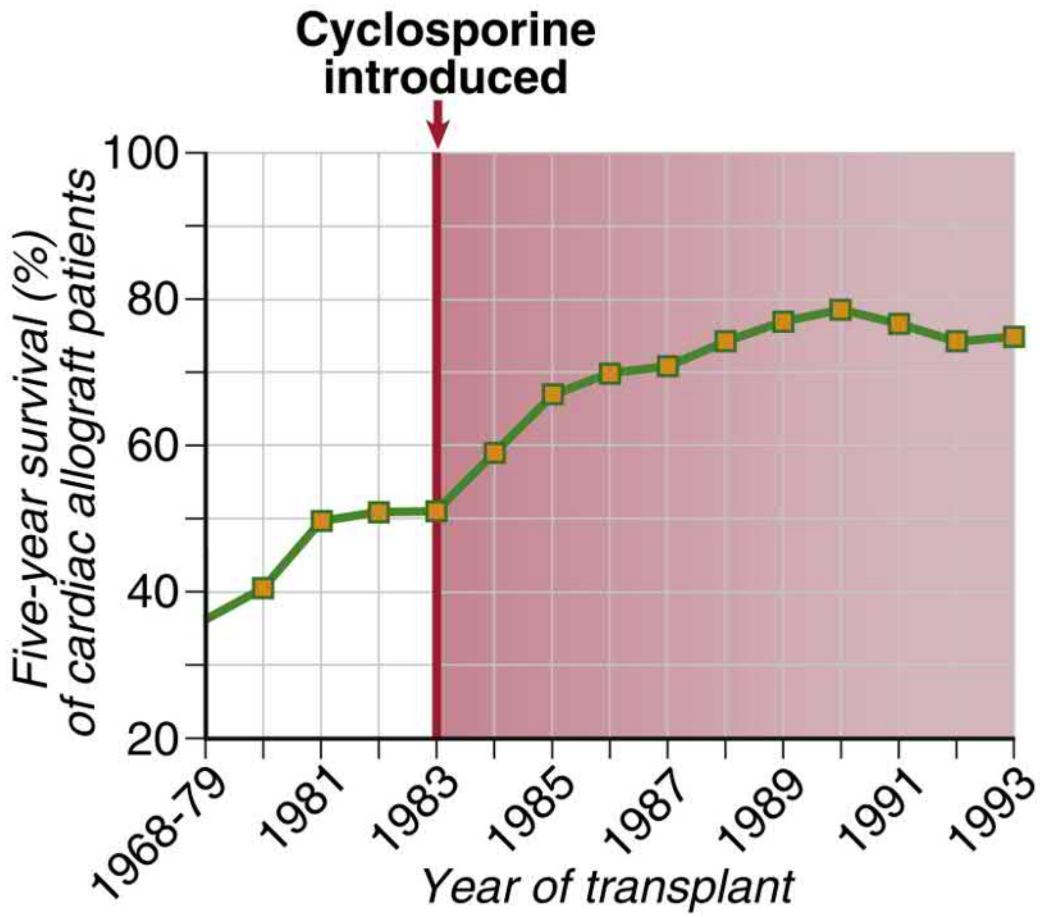


FIGURE 17.13 Influence of cyclosporine on graft survival.Five-year survival rates for patients receiving cardiac allografts increased significantly beginning when cyclosporine was introduced in 1983.

Data from Transplant Patient DataSource, United Network for Organ Sharing, Richmond, Virginia.

Other molecules involved in cytokine and TCR signaling are also targets of immunosuppressive drugs that are in trials for treatment or prevention of allograft rejection. These target molecules include the tyrosine kinase JAK3, which is involved in signaling by various cytokine receptors, including IL-2, and protein kinase C, an essential kinase in TCR signaling.

Antimetabolites

Metabolic toxins that kill proliferating T cells are used in combination with other drugs to treat graft rejection. These agents inhibit the proliferation of lymphocyte precursors during their maturation and also kill proliferating mature T cells that have been stimulated by alloantigens. The first such drug to be developed for the prevention and treatment of rejection was azathioprine. This drug is still used, but it is toxic to precursors of leukocytes in the bone marrow and enterocytes in the gut. The most widely used drug in this class is mycophenolate mofetil (MMF). MMF is metabolized to mycophenolic acid, which blocks the activity of inosine monophosphate dehydrogenase, an enzyme required for de novo synthesis of guanine nucleotides. Because proliferating lymphocytes are particularly dependent on de novo synthesis of purines, MMF targets lymphocytes in a relatively specific manner. MMF is now routinely used, often in combination with tacrolimus, to prevent acute allograft rejection.

Function-Blocking or Depleting Anti-Lymphocyte Antibodies

Antibodies that react with T cell surface structures and deplete or inhibit T cells are used to treat acute rejection episodes. The first anti–T cell antibody used in transplant patients was a mouse monoclonal antibody called OKT3 that is specific for human CD3. (OKT3 was the first monoclonal antibody used as a drug in humans, but it is no longer being produced.) Polyclonal rabbit or horse antibodies specific for a mixture of human T cell surface proteins, so-called anti-thymocyte globulin, also have been in clinical use for many years to treat acute allograft rejection. These anti–T cell antibodies deplete circulating T cells either by activating the complement system to eliminate T cells or by opsonizing them for phagocytosis.

Monoclonal antibodies specific for CD25, the α subunit of the IL-2 receptor are now in clinical use. These reagents prevent T cell activation by blocking IL-2 binding to activated T cells and IL-2 signaling.

Another monoclonal antibody in use in clinical transplantation is one specific for CD52, a cell surface protein expressed widely on most mature B and T cells whose function is not understood. Anti-CD52 (called alemtuzumab) was originally developed to treat B cell tumors, and it was found to profoundly deplete most peripheral B and T cells for many weeks after injection into patients. In the initial clinical trials, it was administered just before and early after transplantation, with the hope that it may induce a prolonged state of graft tolerance, but it is not widely used now.

The major limitation to the use of monoclonal or polyclonal antibodies from other species is that humans given these agents produce anti-Ig antibodies that neutralize the injected foreign Ig. For this reason, humanized antibodies (e.g., against CD3 and CD25), which are less immunogenic, have been developed (see Chapter 5).

Costimulatory Blockade

Drugs that block T cell costimulatory pathways reduce acute allograft rejection. The rationale for the use of these types of drugs is to prevent the delivery of costimulatory signals required for activation of T cells (see Chapter 9). Recall that CTLA4-Ig is a recombinant protein composed of the extracellular portion of CTLA4 fused to an IgG Fc domain. A high-affinity form of CTLA4-Ig, called belatacept, which binds to B7 molecules on APCs and prevents them from interacting with T cell CD28 (see Fig. 9.7), is approved for use in allograft recipients. Clinical studies have shown that belatacept can be as effective as cyclosporine in preventing acute rejection, but its high cost and other factors have limited widespread use of this biologic agent. An antibody that binds to T cell CD40 ligand (CD40L) and prevents its interactions with CD40 on APCs (see Chapter 9) has also proved beneficial for preventing graft rejection in experimental animals. In some experimental protocols, simultaneous blockade of both B7 and CD40 appears to be more effective than either alone in promoting graft survival. However, in clinical trials of anti-CD40L antibody, patients developed thrombotic complications, apparently related to the expression of CD40L on human platelets.

Treatments to Reduce Alloantibodies and Alloreactive B Cells

As we have learned more about the importance of alloantibodies in mediating acute and perhaps chronic rejection, therapies targeting antibodies and B cells that were developed for other diseases are now being used in transplant patients. For example, plasmapheresis is sometimes used to treat acute antibody-mediated rejection. In this procedure, a patient's blood is pumped through a machine that removes the plasma but returns the blood cells to the circulation. In this way, circulating antibodies, including pathogenic alloreactive antibodies, can be removed. Intravenous immunoglobulin (IVIG), which is an accepted therapy for several antibody-mediated inflammatory diseases, is also used to treat acute antibody-mediated rejection. In IVIG therapy, pooled IgG from normal donors is injected intravenously into a patient. The mechanisms of action are not fully understood but likely involve binding of the injected IgG to the patient's Fc receptors on various cell types, thereby reducing alloantibody production and blocking effector functions of the patient's own antibodies. IVIG also enhances degradation of the patient's antibodies by competitively inhibiting their binding to the neonatal Fc receptor (see Chapter 5). B cell depletion by administration of rituximab, an anti-CD20 antibody that is approved for treatment of B cell lymphomas and for autoimmune diseases, is used in some cases of acute antibody-mediated rejection. The proteasome inhibitor bortezomib, which kills plasma cells and is approved to treat multiple myeloma, is also sometimes used to treat antibody-mediated allograft rejection.

Antiinflammatory Drugs

Antiinflammatory agents, specifically corticosteroids, are frequently used to reduce the inflammatory reaction to organ allografts. The proposed mechanism of action of these natural hormones and their synthetic analogs is to block the synthesis and secretion of cytokines, including TNF and IL-1, and other inflammatory mediators, such as prostaglandins, reactive oxygen species, and nitric oxide, produced by macrophages and other inflammatory cells. The net result of this therapy is reduced leukocyte recruitment, inflammation, and graft damage.

Current immunosuppressive protocols have dramatically improved graft survival. Before the use of calcineurin inhibitors, the 1-year survival rate of unrelated cadaveric kidney grafts was between 50% and 60%, with a 90% rate for grafts from living related donors (which are better matched with the recipients). Since cyclosporine, tacrolimus, rapamycin, and MMF have been introduced, the survival rate of unrelated cadaveric kidney grafts has increased to approximately 90% at 1 year. Heart transplantation, for which HLA matching is not practical, has also significantly benefited from the use of the various classes of immunosuppressive drugs reviewed earlier, and now has a similar approximately 90% 1-year survival rate and approximately 75% 5-year survival rate. Experience with other organs is more limited, but survival rates have also improved with modern immunosuppressive therapy, with 10-year patient survival rates of approximately 60% and 75% for pancreas and liver recipients, respectively, and 3-year patient survival rates of 70% to 80% for lung recipients.

Strong immunosuppression is usually started in allograft recipients at the time of transplantation with a combination of drugs called induction therapy. After a few days, the drugs are changed for long-term maintenance of immunosuppression. For example, in the case of adult kidney transplantation, a patient may be initially induced with an anti–IL-2 receptor or anti–T cell depleting antibody and a high-dose corticosteroid and then maintained on a calcineurin inhibitor, an antimetabolite, and maybe low-dose corticosteroids. Acute rejection, when it occurs, is managed by rapidly intensifying immunosuppressive therapy. In modern transplantation, chronic rejection has become a more common cause of allograft failure, especially in cardiac transplantation. Chronic rejection is much less responsive to immunosuppression than is acute rejection.

Immunosuppressive therapy leads to increased susceptibility to infections and tumors. The major goal of immunosuppression to treat graft rejection is to reduce the generation and function of helper T cells and CTLs, which mediate acute cellular rejection. It is therefore not surprising that defense against viruses and other intracellular pathogens and tumors, the physiologic function of T cells, is also compromised in immunosuppressed transplant recipients. A frequent problem in immunosuppressed patients is reactivation of latent herpesviruses, including cytomegalovirus, herpes simplex virus, varicella-zoster virus, and Epstein-Barr virus (EBV). For this reason, transplant recipients are now given prophylactic antiviral therapy for herpesvirus infections. Immunosuppressed allograft recipients are also at greater risk for a variety of opportunistic infections, which normally do not occur in immunocompetent people, including fungal infections (*Pneumocystis jiroveci* pneumonia, histoplasmosis, coccidioidomycosis, and microsporidiosis) and protozoan infections (toxoplasmosis and cryptosporidiosis). Immunosuppressed allograft recipients have a higher risk for development of cancer compared with the general population, including various forms of skin cancer. Some of the tumors that are more frequently found in allograft recipients are known to be caused by viruses, and therefore they may arise because of impaired antiviral immunity. These include uterine cervical carcinoma, which is related to human papillomavirus infection, and lymphomas caused by EBV infection. The lymphomas found in allograft recipients are called post-transplantation lymphoproliferative disorders (PTLDs), and most are derived from EBV-infected B lymphocytes.

Despite the risk for infections and neoplasms associated with the use of immunosuppressive drugs, the major limitation of most of these drugs, including calcineurin inhibitors, mTOR inhibitors, antimetabolites, and corticosteroids, is direct toxicity to cells unrelated to immunosuppression. In some cases, the toxicities affect the same cells as rejection does, such as cyclosporine toxicity to renal tubular epithelial cells, which can complicate the interpretation of declining renal function in kidney allograft recipients.

Methods to Induce Donor-Specific Tolerance

Allograft rejection may be prevented by making the host tolerant to the alloantigens of the graft. Tolerance in this setting means that the host immune system does not injure the graft despite the withdrawal of immunosuppressive agents. It is presumed that tolerance to an allograft will involve the same mechanisms that are involved in tolerance to self antigens (see Chapter 15), that is, anergy, deletion, and active suppression of alloreactive T cells by Tregs. Tolerance is desirable in transplantation because it could be alloantigen specific and would therefore avoid the major problems associated with nonspecific immunosuppression, namely immune deficiency leading to increased susceptibility to infection and development of tumors and drug toxicity. In addition, achieving graft tolerance may reduce chronic rejection, which has to date been unaffected by the commonly used immunosuppressive agents that prevent and reverse acute rejection episodes.

Various experimental approaches and clinical observations have shown that it should be possible to achieve tolerance to allografts. In experiments in mice, Medawar and colleagues found that if neonatal mice of one strain (the recipient) are given spleen cells of another strain (the donor), the recipients will subsequently accept skin grafts from the donor. Such tolerance is alloantigen specific because the recipients will reject grafts from mouse strains that express MHC alleles that differ from those of the spleen cell donor. Renal transplant patients who have received blood transfusions containing allogeneic leukocytes have a lower incidence of acute rejection episodes than do those who have not been transfused. The postulated explanation for this effect is that the introduction of allogeneic leukocytes by transfusion produces tolerance to alloantigens. One underlying mechanism for tolerance induction may be that the transfused donor cells contain immature DCs, which induce unresponsiveness to donor alloantigens. Indeed, pretreatment of potential recipients with blood transfusions is now used as prophylactic therapy to reduce rejection.

Several strategies are being tested to induce donor-specific tolerance in allograft recipients.

- ***Costimulatory blockade.*** It was postulated that recognition of alloantigens in the absence of costimulation would lead to T cell tolerance, and there is some experimental evidence in animals to support this. However, the clinical experience with agents that block costimulation is that they suppress immune responses to the allograft but do not induce long-lived tolerance, and patients have to be maintained on the therapy.
- ***Hematopoietic chimerism.*** We mentioned earlier that transfusion of donor blood cells into the graft recipient inhibits rejection. If the transfused donor cells or progeny of the cells survive for extended periods in the recipient, the recipient becomes a chimera. Long-term allograft tolerance by hematopoietic chimerism has been achieved in a small number of renal allograft recipients who received an HSC transplant from the donor at the same time as the organ allograft, but the risks of HSC transplantation and the availability of appropriate donors may limit the applicability of this approach.
- ***Transfer or induction of Tregs.*** Attempts to generate donor-specific Tregs in culture and to transfer these into graft recipients are ongoing. There has been some success reported in recipients of HSC transplants, in whom infusions of Tregs reduce GVHD.

In rare cases, some transplants survive and function even after immunsuppression is stopped or significantly reduced because of infections or toxicities. This has been most frequently observed in liver transplant patients. Clinicians use the term operational tolerance to refer to this phenomenon. It is not clear in most cases if alloreactive T cell responses are reduced or extinguished. It is also not known why this occurs most often with liver transplantation.

Xenogeneic Transplantation

The use of solid organ transplantation as a clinical therapy is greatly limited by the inadequate numbers of donor organs available. For this reason, the possibility of transplantation of organs from other mammals, such as pigs, into human recipients has kindled great interest.

A major immunologic barrier to xenogeneic transplantation is the presence of natural antibodies in the human recipients that cause hyperacute rejection. More than 95% of primates have natural IgM antibodies that are reactive with carbohydrate determinants expressed by cells of species that are evolutionarily distant, such as pigs, which have organs that are anatomically compatible with humans. The majority of human anti-pig natural antibodies are directed at a carbohydrate determinant formed by the action of a pig α-galactosyltransferase enzyme. This enzyme places an α-linked galactose moiety on the same substrate that in human and other primate cells is fucosylated to form the blood group H antigen. Investigators have produced α-galactosyltransferase gene knockout pigs to try to circumvent this problem, but this strategy alone has not been successful. Humans rarely produce natural antibodies against carbohydrate determinants of closely related species, such as chimpanzees. Thus, organs from chimpanzees or other higher primates might theoretically be accepted in humans. However, ethical and logistic concerns have limited such procedures.

Natural antibodies against xenografts induce hyperacute rejection by the same mechanisms as those seen in hyperacute allograft rejection. These mechanisms include the generation of endothelial cell procoagulants and platelet-aggregating substances, coupled with the loss of endothelial anticoagulant mechanisms. However, the consequences of activation of human complement on pig cells are typically more severe than the consequences of activation of complement by natural antibodies on human allogeneic cells. This may be because some of the complement regulatory proteins made by pig cells are not able to interact with human complement proteins and thus cannot limit the extent of injury induced by the human complement system (see Chapter 13). For these reasons, investigators have developed genetically modified pigs that are transgenic for human complement regulatory proteins. The CRISPR-Cas9 gene editing technology is now being applied to generate pigs with multiple genetic modifications chosen to reduce xenograft rejection.

Even when hyperacute rejection is prevented, xenografts are often damaged by a form of acute vascular rejection that occurs within 2 to 3 days of transplantation. This form of rejection has been called delayed xenograft rejection, accelerated acute rejection, or acute vascular rejection and is characterized by intravascular thrombosis and necrosis of vessel walls. The mechanisms of delayed xenograft rejection are incompletely understood; recent findings indicate that there may be incompatibilities between primate platelets and porcine endothelial cells that promote thrombosis independent of antibody-mediated damage.

Xenografts also can be rejected by T cell–mediated immune responses to xenoantigens. The mechanisms of cell-mediated rejection of xenografts are thought to be similar to those that we have described for allograft rejection.

Blood Transfusion and the Abo and Rh Blood Group Antigens

Blood transfusion is a form of transplantation in which whole blood or blood cells from one or more individuals are transferred intravenously into the circulation of another individual. Blood transfusions are most often performed to replace blood lost by hemorrhage or to correct defects caused by inadequate production of blood cells, which may occur in a variety of diseases. The major barrier to successful blood transfusions is the immune response to cell surface molecules that differ among individuals. The most important alloantigen system in blood transfusion is the ABO system, which we will discuss in detail later. Individuals who do not express a particular blood group antigen produce natural IgM antibodies against that antigen. If such individuals are given blood cells expressing that antigen, the preexisting antibodies bind to the transfused cells, activate complement, and cause transfusion reactions, which can be life-threatening. Transfusion across an ABO barrier may trigger an immediate hemolytic reaction, resulting in both intravascular lysis of red blood cells, probably mediated by the complement system, and extensive phagocytosis of antibody- and complement-coated erythrocytes by macrophages in the liver and spleen. Hemoglobin is liberated from the lysed red blood cells in quantities that may be toxic for kidney cells, causing acute renal tubular cell necrosis and kidney failure. High fever, shock, and disseminated intravascular coagulation may also develop, suggestive of release of massive amounts of cytokines (e.g., TNF or IL-1). The disseminated intravascular coagulation consumes clotting factors faster than they can be synthesized, and the patient may paradoxically die of bleeding in the presence of widespread clotting. More delayed hemolytic reactions may result from incompatibilities of minor blood group antigens. These result in progressive loss of the transfused red blood cells, leading to anemia and jaundice, the latter a consequence of overloading the liver with hemoglobin-derived pigments.

ABO Blood Group Antigens

The ABO antigens are carbohydrates, linked to cell surface proteins and lipids, which are synthesized by polymorphic glycosyltransferase enzymes that vary in activity depending on the inherited allele (Fig. 17.14) **.** The ABO antigens were the first alloantigen system to be defined in mammals. These specific carbohydrate antigens are present on red blood cells, hence their name, but they are also found on endothelial and some epithelial cells. All normal individuals produce a common core glycan, which is attached mainly to plasma membrane proteins. Most individuals possess a fucosyltransferase that adds a fucose moiety to a nonterminal sugar residue of the core glycan, and the fucosylated glycan is called the H antigen. A single gene on chromosome 9 encodes a glycosyltransferase enzyme that may further modify the H antigen. There are three allelic variants of this gene. The O allele gene product is devoid of enzymatic activity. The A allele–encoded enzyme transfers a terminal N-acetylgalactosamine moiety onto the H antigen, and the B allele gene product transfers a terminal galactose moiety. Individuals who are homozygous for the O allele cannot attach terminal sugars to the H antigen and express only the H antigen. In contrast, individuals who possess an A allele (AA homozygotes, AO heterozygotes, or AB heterozygotes) form the A antigen by adding terminal N-acetylgalactosamine to some of their H antigens. Similarly, individuals who express a B allele (BB homozygotes, BO heterozygotes, or AB heterozygotes) form the B antigen by adding terminal galactose to some of their H antigens. AB heterozygotes form both A and B antigens from some of their H antigens. The terminology has been simplified so that OO individuals are said to be blood type O; AA and AO individuals are blood type A; BB and BO individuals are blood type B; and AB individuals are blood type AB. Mutations in the gene encoding the fucosyltransferase that produces the H antigen are rare; people who are homozygous for such a mutation are said to have the Bombay blood group and cannot produce H, A, or B antigens.

Individuals who express a particular A or B blood group antigen are tolerant to that antigen, but individuals who do not express that antigen produce natural antibodies that recognize the antigen. Almost all individuals express the H antigen and, therefore, are tolerant to this antigen and do not produce anti-H antibodies. Individuals who express A or B antigens are tolerant to these molecules and do not produce anti-A or anti-B antibodies, respectively. However, blood group O and A individuals produce anti-B IgM antibodies, and blood group O and B individuals produce anti-A IgM antibodies. Individuals with the Bombay phenotype who are unable to produce the core H antigens make antibodies against H, A, and B antigens. On face value, it seems paradoxical that individuals who do not express a blood group antigen make antibodies against it. The likely explanation is that the antibodies are produced against glycolipids of intestinal bacteria that happen to cross-react with the ABO antigens, unless the individual is tolerant to one or more of these. Predictably, the presence of any blood group antigen on an individual's red blood cells induces tolerance to that antigen.

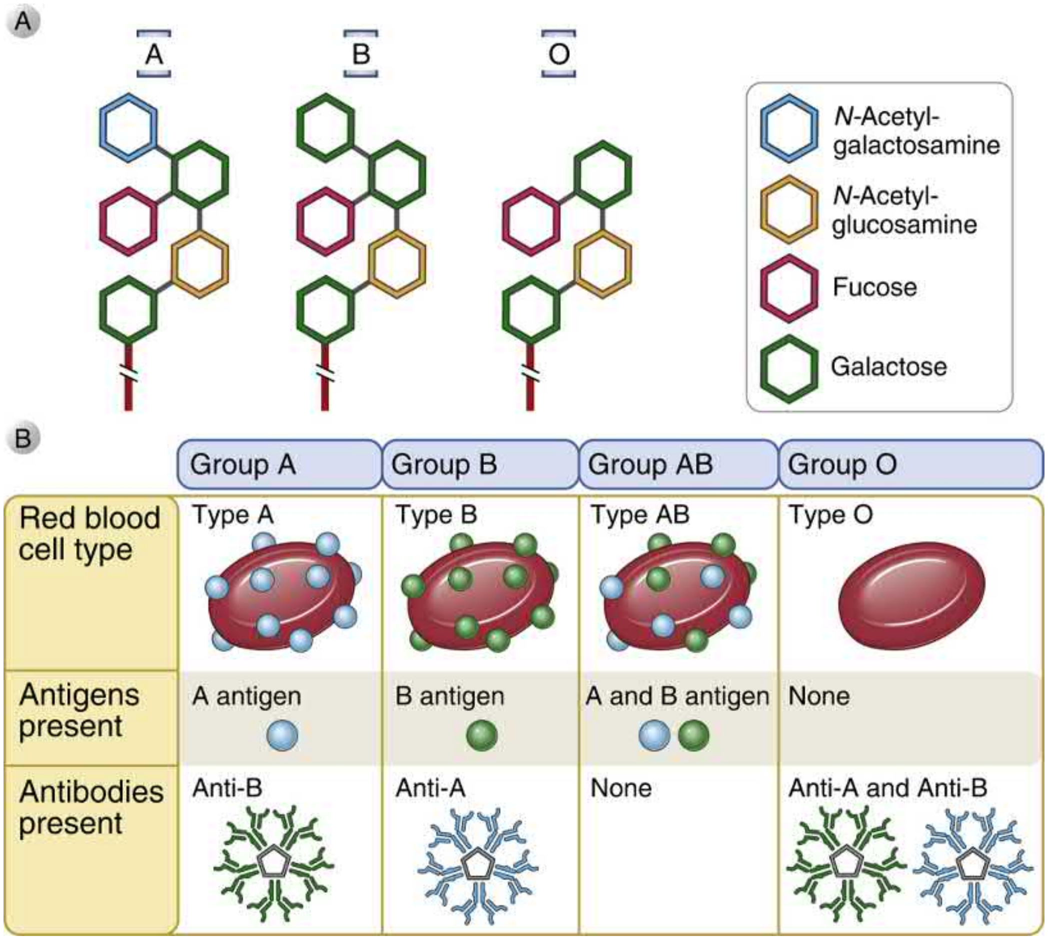

A
A
B
O
N-Acetyl-galactosamine
N-Acetyl-glucosamine
Fucose
Galactose
B
Group A
Group B
Group AB
Group O
Red blood cell type
Type A
Type B
Type AB
Type O
Antigens present
A antigen
B antigen
A and B antigen
None
Antibodies present
Anti-B
Anti-A
None
Anti-A and Anti-B

FIGURE 17.14 ABO blood group antigens. **A,** Blood group antigens are carbohydrate structures added onto proteins or lipids by the action of glycosyltransferases (see text). **B,** Different blood group antigens are produced by the addition of different sugars by different inherited glycosyltransferases. Individuals who express a particular blood group antigen are tolerant to that antigen but produce natural antibodies that react with other blood group antigens.

In clinical transfusion, the choice of blood donors for a particular recipient is based on the expression of blood group antigens and the antibody responses to them. If a patient receives a transfusion of red blood cells from a donor who expresses the antigen not expressed on self red blood cells, a transfusion reaction may result (described earlier). It follows that AB individuals can tolerate transfusions from all potential donors and are therefore called universal recipients; similarly, O individuals can tolerate transfusions only from O donors but can provide blood to all recipients and are therefore called universal donors. In general, differences in minor blood groups lead to red blood cell lysis only after repeated transfusions trigger a secondary antibody response.

A and B blood group antigens are expressed on many other cell types in addition to blood cells, including endothelial cells. For this reason, ABO typing is critical to avoid hyperacute rejection of certain solid organ allografts, as discussed earlier in the chapter. ABO incompatibility between mother and fetus generally does not cause problems for the fetus because most of the anti-carbohydrate antibodies are IgM and do not cross the placenta.

Other Blood Group Antigens

Lewis Antigen

The same glycoproteins that carry the A and B blood group determinants can be modified by other glycosyltransferases to generate minor blood group antigens. For example, addition of fucose moieties at other nonterminal positions can be catalyzed by different fucosyltransferases and create epitopes of the Lewis antigen system. Lewis antigens have received much attention from immunologists because these carbohydrate groups serve as ligands for E-selectin and P-selectin and thus play a role in leukocyte migration (see Chapter 3). They are not, however, involved in strong transfusion reactions because individuals do not make natural antibodies against them.

Rhesus Antigen

The Rhesus (Rh) antigens, named after the monkey species in which they were originally identified, are another clinically important set of blood group antigens. Rh antigens are nonglycosylated, hydrophobic cell surface proteins found in red blood cell membranes and are structurally related to other red blood cell membrane proteins with transporter functions. Rh proteins are encoded by two tightly linked and highly homologous genes, but only one of them, called RhD, is commonly considered in clinical blood typing. This is because up to 15% of the population has a deletion or other alteration of the RhD allele. These people, called Rh negative, are not tolerant to the RhD antigen and will make antibodies to the antigen if they are exposed to Rh-positive blood cells.

The major clinical significance of anti-Rh antibodies is related to hemolytic reactions in developing fetuses that are similar to transfusion reactions. Rh-negative mothers carrying an Rh-positive fetus can be sensitized by fetal red blood cells that enter the maternal circulation, usually during childbirth. Because the Rh antigen is a protein, as opposed to the carbohydrate ABO antigens, class-switched high-affinity IgG antibodies specific for Rh are produced in Rh-negative mothers. Subsequent pregnancies in which the fetus is Rh positive are at risk because the maternal anti-Rh IgG antibodies can cross the placenta and mediate the destruction of the fetal red blood cells. This causes **hemolytic disease of the fetus and newborn** (also called erythroblastosis fetalis) and can be lethal for the fetus. This disease can be prevented by administration of anti-RhD antibodies to the mother within 72 hours of birth of the first Rh-positive baby. The treatment prevents the baby's Rh-positive red blood cells that entered the mother's circulation from inducing the production of anti-Rh antibodies in the mother. The exact mechanisms of action of the administered antibodies are not clear but may include phagocytic clearance or complement-mediated lysis of the baby's red blood cells before they can elicit an antibody response in the mother, or Fc receptor–dependent feedback inhibition of the mother's RhD-specific B cells (see Chapter 12).

Hematopoietic Stem Cell (HSC) Transplantation

HSC transplantation is a clinical procedure to treat diseases caused by intrinsic defects in one or more hematopoietic lineages and more commonly to treat cancers of blood cells. In this procedure, the patient's own hematopoietic cells are destroyed and HSCs from a healthy donor are then given to the patient. We consider HSC transplantation separately from other forms of transplantation because this type of grafting has several unique features that are not encountered with solid organ transplantation.

Indications, Methods, and Immune Barriers in Hematopoietic Stem Cell Transplantation

The transplantation of pluripotent HSCs was done in the past using an inoculum of bone marrow cells collected by aspiration, and the procedure is often called bone marrow transplantation. In modern clinical practice, HSCs are more often obtained from the blood of donors, after treatment of the donor with colony-stimulating factors that mobilize stem cells from the bone marrow. The recipient is treated before transplantation with a combination of chemotherapy, immunotherapy, or irradiation to free up niches for the transferred stem cells and, in the case of treating hematopoietic malignancies, to kill as many of the cancer stem cells as possible. After transplantation, the injected stem cells repopulate the recipient's bone marrow and differentiate into all of the hematopoietic lineages.

HSC transplantation is most often used clinically in the treatment of leukemias and pre-leukemic conditions. The mechanism by which HSC transplantation cures hematopoietic neoplasms is in part the graft-versus-tumor effect, in which mature T cells present in the bone marrow or stem cell inoculum recognize alloantigens on residual tumor cells and destroy them. NK cells in the injected HSC inoculum may also recognize and kill leukemic cells. Autologous HSC transplantation is used to treat the plasma cell–derived tumor called myeloma. Because many of the neoplastic cells are in the patient's bone marrow, these are killed when the bone marrow is ablated as part of the conditioning regimen. The HSCs that are transplanted are from the same patient but they do not contain myeloma cells because these cells do not circulate in the blood. Thus, the function of the bone marrow is restored and the tumor cells are eliminated. Since the transplant is autologous, there is no risk for its rejection or GVHD (described later).

HSC transplantation is also used clinically to treat diseases caused by inherited mutations in genes affecting only cells derived from HSCs, such as lymphocytes or red blood cells. Examples of such diseases that can be treated by HSC transplantation are X-linked severe combined immunodeficiency disease (X-SCID), IPEX (the disease caused by FOXP3 mutations and a deficiency of regulatory T cells), and hemoglobin mutations, such as beta-thalassemia major and sickle cell disease. X-SCID has also been treated by expressing the normal gene in the patient's own HSCs and transplanting them to correct the deficiency. The same approach is applicable to other diseases in which the defective gene is known and is functional in blood cells, which are produced from HSCs. Because these transplants are autologous, they do not cause immunologic problems.

Allogeneic HSCs are rejected by even a minimally immunocompetent host, and therefore the donor and recipient must be carefully matched at all MHC loci. The mechanisms of rejection of HSCs are not completely known, but in addition to adaptive immune mechanisms, HSCs may be rejected by NK cells. The role of NK cells in bone marrow rejection has been studied in experimental animals. Irradiated F1 hybrid mice reject bone marrow cells donated by either inbred parent. This phenomenon, called hybrid resistance, appears to violate the classical laws of solid-organ transplantation (in which F1 mice do not react against grafts from either parent, see Fig. 17.3). Hybrid resistance is seen in T cell–deficient mice, and depletion of recipient NK cells with anti–NK cell antibodies prevents the rejection of parental bone marrow cells. Hybrid resistance is probably due to host NK cells reacting against bone marrow precursors that lack class I MHC molecules expressed by the host. Recall that, normally, recognition of self class I MHC inhibits the activation of NK cells, and, if these self MHC molecules are missing, the NK cells are released from inhibition (see Fig. 4.10).

Even after successful engraftment, two additional problems are frequently associated with HSC transplantation, that is, GVHD and immunodeficiency, which are discussed next.

Immunologic Complication of Hematopoietic Stem Cell Transplantation

Graft-Versus-Host Disease

GVHD is caused by the reaction of grafted mature T cells in the HSC inoculum with alloantigens of the host. It occurs when the host is immunocompromised and therefore unable to reject the allogeneic cells in the graft. In most cases, the reaction is directed against minor histocompatibility antigens of the host because bone marrow transplantation is not usually performed when the donor and recipient have differences in MHC alleles. GVHD may also develop when solid organs that contain significant numbers of T cells are transplanted, such as the small bowel, lung, or liver.

GVHD is a major limitation to the success of bone marrow transplantation. Immediately after HSC transplantation, immunosuppressive agents, including the calcineurin inhibitors cyclosporine and tacrolimus, antimetabolites such as methotrexate, and the mTOR inhibitor sirolimus, are given for prophylaxis against the development of GVHD. Despite these aggressive prophylactic strategies, GVHD is the principal cause of mortality among HSC transplant recipients. GVHD may be classified on the basis of histologic patterns into acute and chronic forms.

Acute GVHD is characterized by epithelial cell death in the skin (Fig. 17.15A-B), liver (mainly the biliary epithelium), and gastrointestinal tract. It is manifested clinically by rash, jaundice, diarrhea, and gastrointestinal hemorrhage. When the epithelial cell death is extensive, the skin or the lining of the gut may slough off. In this circumstance, acute GVHD may be fatal.

Chronic GVHD is characterized by fibrosis and atrophy of one or more of the same organs, without evidence of acute cell death (Fig. 17.15C). Chronic GVHD may also involve the lungs and produce obliteration of small airways, called bronchiolitis obliterans, similar to what is seen in chronic rejection of lung allografts. When it is severe, chronic GVHD leads to complete dysfunction of the affected organ.

In animal models, acute GVHD is initiated by mature T cells transferred with HSCs, and elimination of mature donor T cells from the graft can prevent the development of GVHD. In clinical HSC transplantation, efforts to eliminate T cells from the inoculum have reduced the incidence of GVHD but also decreased the graft-versus-leukemia effect that is often critical in treating leukemias by this type of transplantation. T cell–depleted HSC preparations also tend to engraft poorly, perhaps because mature T cells produce colony-stimulating factors that aid in stem cell repopulation.

Although GVHD is initiated by grafted T cells recognizing host alloantigens, the effector cells that cause epithelial cell injury are less well defined. On histologic examination, NK cells are often attached to the dying epithelial cells, suggesting that NK cells are important effector cells of acute GVHD. $CD8^+$ CTLs and cytokines also appear to be involved in tissue injury in acute GVHD.

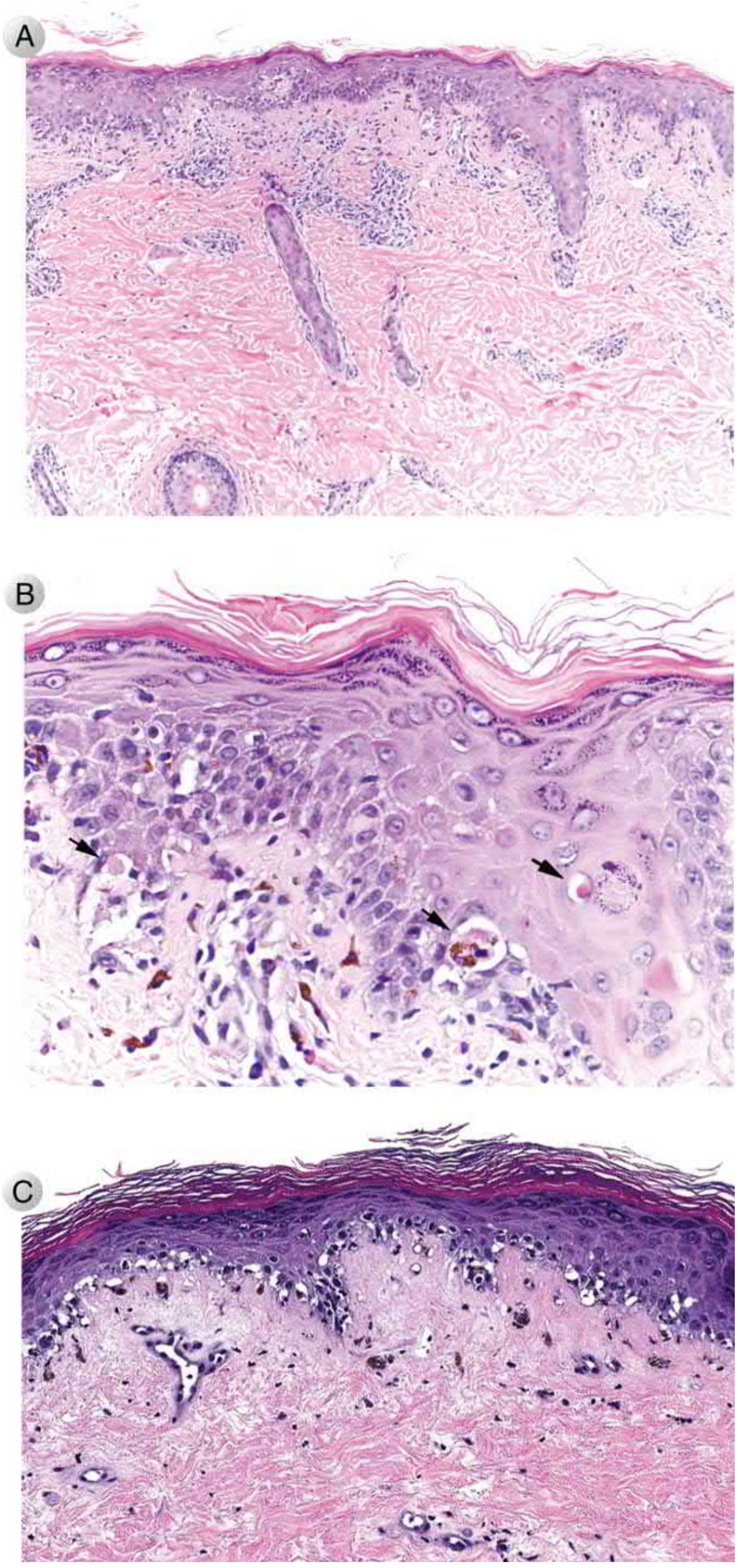

A
B
C

FIGURE 17.15 Histopathology of graft-versus-host disease in the skin. *Acute GVHD*. Low-power **(A)** and high-power **(B)** photomicrographs are shown of a skin biopsy from a patient with acute graft-versus-host disease *(GVHD)*. A sparse lymphocytic infiltrate can be seen at the dermal-epidermal junction, and damage to the epithelial layer is indicated by spaces at the dermal-epidermal junction (vacuolization), cells with abnormal keratin staining (dyskeratosis), apoptotic keratinocytes *(arrows),* and disorganization of maturation of keratinocytes from the basal layer to the surface. *Chronic GVHD*. **C,** Chronic GVHD showing a sparse lymphocytic infiltrate at the dermal-epidermal junction, which has resulted in occasional damaged keratinocytes. The epidermis is thinned, signifying atrophy. The underlying dermis shows thickening of collagen bundles, indicative of sclerosis.

A and *B,* Courtesy Dr. Scott Grantor, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts. *C,* Courtesy Dr. Jarish Cohen, Department of Pathology, University of California San Francisco.

The relationship of chronic GVHD to acute GVHD is not known and raises issues similar to those of relating chronic allograft rejection to acute allograft rejection. For example, chronic GVHD may represent the fibrosis of wound healing secondary to acute loss of epithelial cells. However, chronic GVHD can arise without evidence of prior acute GVHD. An alternative explanation is that chronic GVHD represents a response to ischemia caused by vascular injury.

Both acute and chronic GVHD are commonly treated with intense immunosuppression, such as high doses of corticosteroids, but many patients do not respond well. Therapeutic failures may be because these treatments target only some of many effector mechanisms at play in GVHD, and some treatments may deplete Tregs, which are important for preventing GVHD. The BTK (Bruton tyrosine kinase) inhibitor ibrutinib, which had been previously approved for treating B cell malignancies, also has been shown in clinical trials to be effective in treating chronic GVHD, and has been approved by the U.S. Food and Drug Administration for this purpose. Therapies in development include Treg cell transfer.

Immunodeficiency After Hematopoietic Stem Cell Transplantation

HSC transplantation is often accompanied by immunodeficiency. Several factors may contribute to defective immune responses in recipients. The transplant recipients may be unable to regenerate a complete new lymphocyte repertoire. Radiation therapy and chemotherapy used to prepare recipients for transplantation may deplete the patient’s memory cells and long-lived plasma cells, and it can take a long time to regenerate these populations.

The consequence of immunodeficiency is that HSC transplant recipients are susceptible to viral infections, especially reactivation of cytomegalovirus infection, and to many bacterial and fungal infections. They are also susceptible to EBV-provoked B cell lymphomas. The immune deficiencies of HSC transplant recipients can be more severe than those of conventionally immunosuppressed patients. Therefore, the recipients commonly receive prophylactic antibiotics, antiviral prophylaxis to prevent cytomegalovirus infections, and antifungal prophylaxis to prevent invasive *Aspergillus* infection. Recipients are also immunized against common infections, to restore the protective immunity that is lost because of ablation of the bone marrow before HSC transplantation.

There is great interest in the use of pluripotent stem cells to repair tissues that have little natural regenerative capacity, such as cardiac muscle, brain, and spinal cord. One approach is to use embryonic stem cells, which are pluripotent stem cells derived from the blastocyst stage of human embryos. Although embryonic stem cells have not yet been widely used clinically, it is likely that a major barrier to their successful grafting will be their alloantigenicity and rejection by the recipient's immune system. A possible solution to this may be to use induced pluripotent stem (iPS) cells, which can be derived from adult somatic tissues by transduction of certain genes. The immunologic advantage of the iPS cell approach is that these cells can be derived from somatic cells harvested from the patient, and therefore they will not be rejected. However, the current experience is that iPS cells do not efficiently generate mature, stable cell populations in vivo, so their potential for cell replacement may be limited. Another solution being investigated is to remove MHC genes from allogeneic embryonic stem cells by CRISPR-Cas9 genome editing technology.

Summary

- Allografts are tissues or organs transplanted from one individual to a genetically nonidentical recipient. Allografts stimulate a specific immune response called rejection that can destroy the graft. The major molecular targets in allograft rejection are allogeneic class I and class II major histocompatibility complex (MHC) molecules.
- Intact allogeneic MHC molecules may be presented on donor antigen-presenting cells (APCs) to recipient T cells (direct recognition), or the allogeneic MHC molecules may be internalized by host APCs that enter the graft or reside in draining lymphoid organs and be processed and presented to T cells as peptides associated with self MHC molecules (indirect recognition).
- The frequency of T cells capable of recognizing allogeneic MHC molecules is very high, compared with T cells that recognize any microbial peptide bound to self MHC, explaining why the response to alloantigens is much

stronger than the response to conventional foreign antigens.

- Graft rejection is mediated by T cells, including cytotoxic T lymphocytes that kill graft cells and helper T cells that cause cytokine-mediated inflammation resembling delayed-type hypersensitivity reactions, and by antibodies.
- Several effector mechanisms cause rejection of solid organ grafts. Preexisting antibodies specific for donor blood group, MHC, or other antigens cause hyperacute rejection characterized by thrombosis of graft vessels. Alloreactive T cells and antibodies produced in response to the graft cause blood vessel wall damage and parenchymal cell death, called acute rejection. Chronic rejection is characterized by fibrosis and arterial stenosis (graft vasculopathy), which may be due to inflammatory reactions mediated by T cell cytokines.
- Graft rejection may be prevented by minimizing the immunogenicity of the graft (by limiting MHC allelic differences) and treated by immunosuppression. Most immunosuppression is directed at T cell responses and entails the use of cytotoxic drugs, specific immunosuppressive agents, and anti–T cell antibodies. Widely used immunosuppressive agents target calcineurin, mTOR (mechanistic target of rapamycin), and lymphocyte DNA synthesis. Immunosuppression is often combined with antiinflammatory drugs, such as corticosteroids, that inhibit cytokine synthesis by macrophages and other cells. Patients receiving solid organ transplants may become immunodeficient because of their therapy and are susceptible to viral infections and malignant tumors.
- Xenogeneic transplantation of solid organs from pigs into humans is limited by the presence of natural antibodies to carbohydrate antigens on the cells of discordant species that cause hyperacute rejection. Other mechanisms of xenograft failure include antibody-mediated acute vascular rejection, T cell–mediated immune response to xenogeneic

MHC molecules, and prothrombotic effects of xenogeneic endothelium on human platelets and coagulation proteins.

- The ABO blood group antigens are polymorphic carbohydrate structures present on blood cells and endothelium that limit transfusions and some solid organ transplantations between individuals. Preexisting natural anti-A or anti-B IgM antibodies are present in individuals who do not express A or B antigens on their cells, respectively, and these antibodies can cause transfusion reactions and hyperacute allograft rejection.
- Rhesus (Rh) antigens are proteins on red blood cells that can stimulate IgG antibody responses in Rh-negative women carrying Rh-positive fetuses, and these anti-Rh antibodies can cause hemolytic disease in Rh-positive fetuses during subsequent pregnancies.
- Hematopoietic stem cell (HSC) transplants are performed to treat leukemias and genetic defects restricted to hematopoietic cells. HSC transplants are susceptible to rejection, and recipients require intense preparatory immunosuppression. In addition, T lymphocytes and NK cells in the HSC grafts may respond to alloantigens of the host and cause graft-versus-host disease (GVHD). Acute GVHD is characterized by epithelial cell death in the skin, intestinal tract, and liver; it may be fatal. Chronic GVHD is characterized by fibrosis and atrophy of one or more of these same target organs and the lungs and also may be fatal. HSC transplant recipients also often develop severe immunodeficiency, rendering them susceptible to infections.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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∗Thomas E.D, Lochte Jr. H.L, Lu W.C, Ferrebee J.W. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med* . 1957;257:491–496 *(The report of the first successful bone marrow transplant to treat leukemeia, which involved a donor and recipient who were identical twins. Thomas received the Nobel Prize for this work. See https://www.nobelprize.org/prizes/medicine/1990/murray/lecture/.).*

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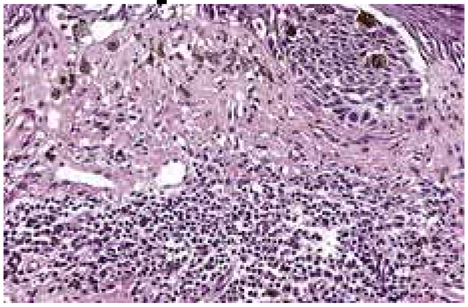
Chapter 18: Tumor Immunology



Overview of Tumor Immunity,

Tumor Antigens,
  Neoantigens: Antigens Encoded by Mutated Genes,
  Antigens of Oncogenic Viruses,
  Overexpressed Cellular Proteins,
  Other Antigens of Tumors,

Immune Responses To Tumors,
  T Lymphocytes,
  Antibodies,
  Natural Killer Cells,
  Macrophages,
  The Role of Innate and Adaptive Immunity in Promoting Tumor Growth,

Evasion of Immune Responses by Tumors,
  Molecules and Cells That Inhibit Immune Responses to Tumors,

  - Loss of Tumor Antigen Expression,
- Immunotherapy For Tumors,
  - Passive Immunotherapy With Monoclonal Antibodies or Antibody-Like Molecules,
  - Adoptive Cellular Therapy With Antitumor T Cells,
  - Immune Checkpoint Blockade: Targeting T Cell Inhibitory Pathways,
  - Vaccination With Tumor Antigens,
  - Other Approaches for Stimulating Antitumor Immunity,
- Summary,

Cancer is a major health problem worldwide and one of the most important causes of morbidity and mortality in children and adults. The lethality of malignant tumors is due to their uncontrolled growth and spread within normal tissues, causing damage and functional impairment. The malignant phenotype of cancers results from defective regulation of cell proliferation, resistance of the tumor cells to apoptotic death, and the ability of the tumor cells to invade host tissues and metastasize to distant sites. In addition, reflecting our improved understanding of immune responses against cancers and the therapeutic success of cancer immunotherapy, we now include the ability of tumor cells to evade host immune defense mechanisms as one of the hallmark features of cancer. The concept of **immune surveillance** of cancer, which was proposed by Macfarlane Burnet and Lewis Thomas in the 1950s, states that a physiologic function of the immune system is to recognize and destroy clones of transformed cells before they grow into tumors and to kill tumors after they are formed. The existence of immune surveillance has been demonstrated by the increased incidence of some types of tumors in immunocompromised experimental animals and humans. More recently, we have learned that immune responses against many human cancers are ineffective, but they can be therapeutically stimulated to destroy tumors. In this chapter, we will describe the types of antigens that are expressed by malignant tumors, how the immune system recognizes and responds to these antigens, how tumors evade the host immune system, and the application of immunologic approaches to the treatment of cancer.

Overview of Tumor Immunity

Several characteristics of tumor antigens and immune responses to tumors are fundamental to an understanding of tumor immunity and for the development of strategies for cancer immunotherapy.

Tumor antigens stimulate specific adaptive immune responses that can prevent or limit the growth and spread of the tumors. Clinical studies, pathologic analyses of tumors, and animal experiments have all established that although tumor cells are derived from host cells, the tumors elicit immune responses in their hosts. Most evidence indicates that the clinically relevant immune responses involve T cells, and especially CD8$^+$ cytotoxic T lymphocytes (CTLs). Histopathologic studies show that many tumors are surrounded by mononuclear cell infiltrates composed of T lymphocytes and macrophages and that activated lymphocytes and macrophages are present in lymph node draining the sites of tumor growth (Fig. 18.1A–C). Quantitative analyses of these infiltrates in colon cancers and some other tumor types have revealed that higher numbers of T cells, in particular CD8$^+$ CTLs and memory cells and CD4$^+$ Th1 cells, are associated with a better prognosis than tumors with fewer of these cells (Fig. 18.1D).

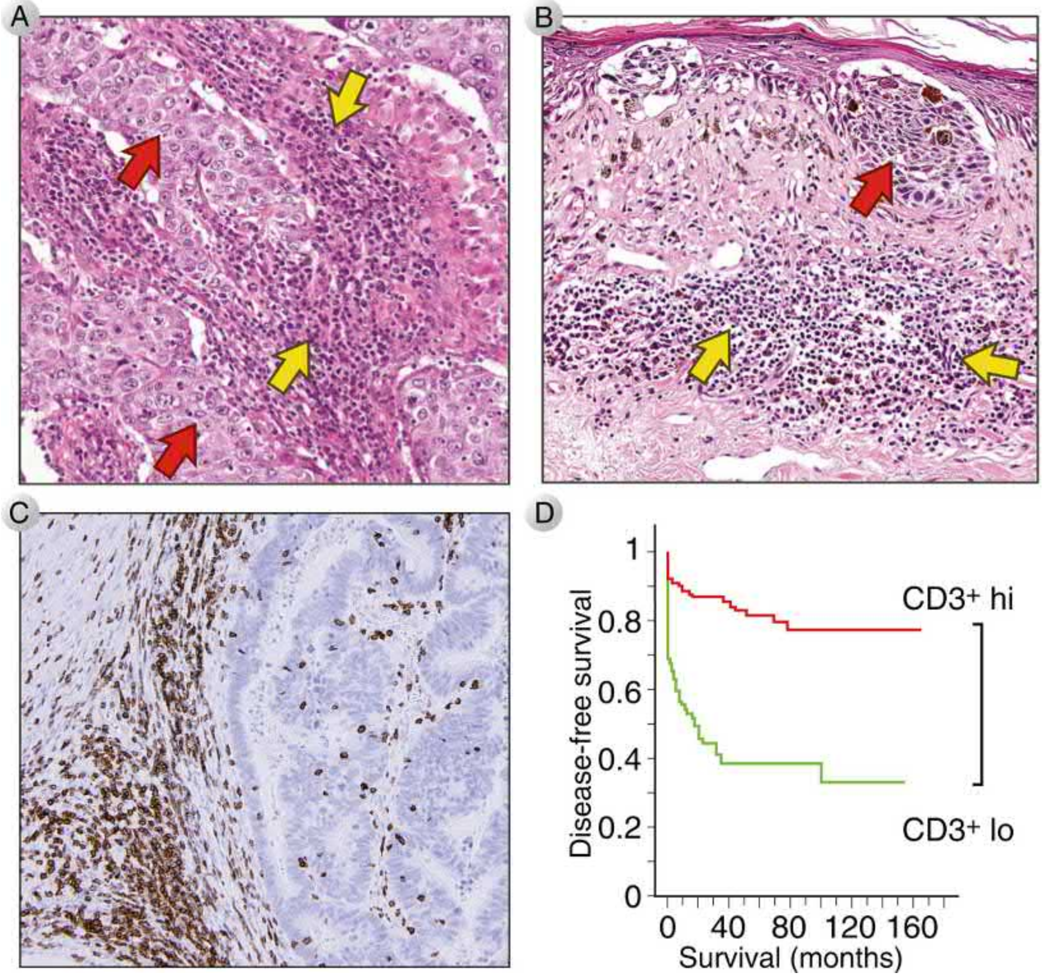

A
B
C
D
1
0.8
0.6
0.4
0.2
0
Disease-free survival
0
40
80
120
160
Survival (months)
CD3+ hi
CD3+ lo

FIGURE 18.1 Lymphocytic inflammation associated with tumors.Certain tumor types more frequently have associated lymphocytic infiltrates, including medullary breast carcinoma **(A)** and malignant melanoma **(B).** *Red arrows* indicate malignant cells. *Yellow arrows* indicate lymphocyte-rich inflammatory infiltrates. Immunohistochemical staining of resected tumors can be used to enumerate different types of T cells associated with the tumor, such as an infiltrate of $CD8^+$ T cells in a colonic carcinoma **(C)**. The tumor cells appear *blue* and the $CD8^+$ T cells appear *brown.* Increased density of $CD3^+$ T cells within the tumor, detected in this way, is associated with longer disease-free survival **(D).**

C, Courtesy Department of Pathology, Brigham and Women's Hospital. *D,* From Galon J, Costes A, Sanchez-Cabo F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science.* 2006;313:1960–1964.

The first experimental demonstration that tumors can induce protective immune responses came from studies of transplanted tumors performed in the 1950s. A sarcoma may be induced in an inbred mouse by painting its skin with the chemical carcinogen methylcholanthrene (MCA). If the MCA-induced tumor is excised and transplanted into other syngeneic mice, the tumor grows. In contrast, if cells from the original tumor are transplanted back into the original host, the mouse rejects this transplant and no tumor grows. The same mouse that has become immune to its own tumor does not reject MCA-induced tumors produced in other mice, which have different MCA-induced mutations and express different tumor antigens. Furthermore, transfer of T cells from the tumor-bearing animal to a tumor-free animal can impart protective immunity against that tumor. Thus, immune responses to these tumors exhibit the defining characteristics of adaptive immunity—that is, specificity, memory, and a key role of lymphocytes. Subsequent work showed that the frequency of spontaneous or MCA-induced tumors in genetically immunodeficient mice is increased compared with immunologically normal mice, further establishing a role of the immune system in tumor immune surveillance. Immunodeficient humans, such as patients with acquired immunodeficiency syndrome (AIDS) or transplant recipients given immunosuppressive drugs, are at increased risk for developing tumors, some of which are caused by viruses (reflecting defective antiviral immunity), but also some tumors not known to have a viral etiology.

Immune responses frequently fail to prevent the growth of tumors. There may be several reasons why antitumor immunity is unable to eradicate cancers. First, many tumors have developed specialized mechanisms for subverting host immune responses. We will return to these inhibitory mechanisms later in the chapter. Second, tumor cells lose the expression of antigens that may be recognized by the host immune system. Even tumors that do elicit effective immune responses may become less immunogenic over time because subclones that do not express immunogenic antigens have a selective survival advantage. Third, the rapid growth and spread of a tumor may overwhelm the capacity of the immune system to effectively control the tumor, which requires that all the malignant cells be eliminated.

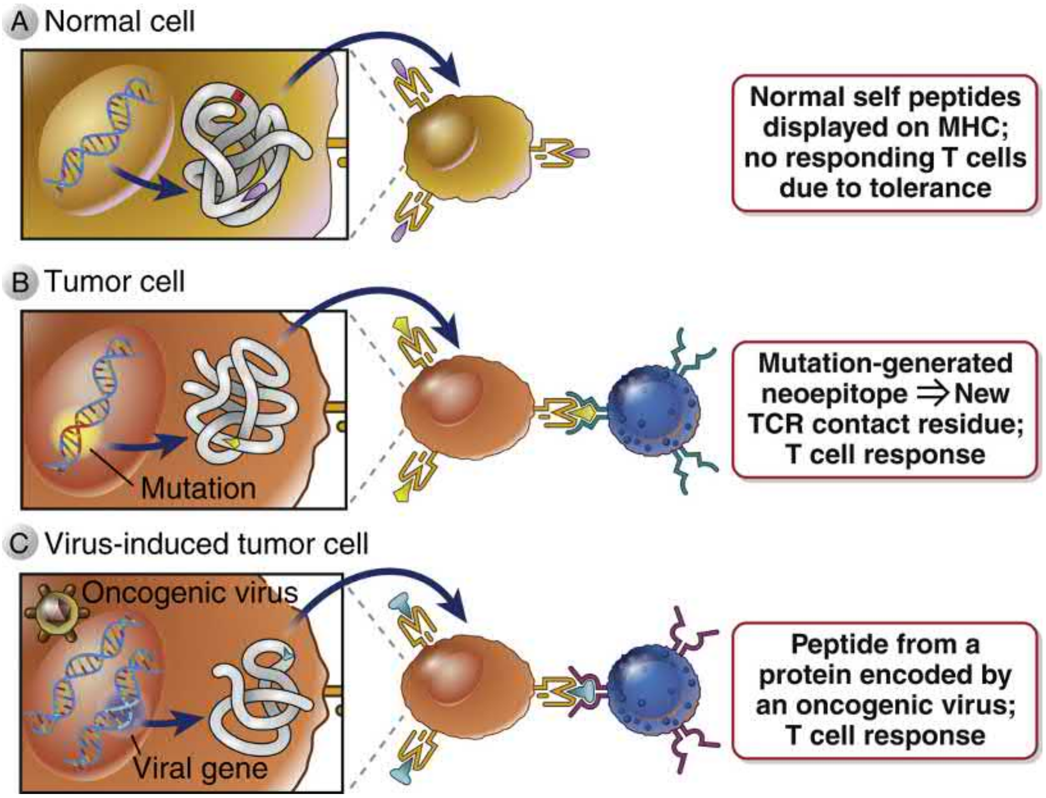

A Normal cell
Normal self peptides displayed on MHC; no responding T cells due to tolerance
B Tumor cell
Mutation
Mutation-generated neoepitope ⇒ New TCR contact residue; T cell response
C Virus-induced tumor cell
Oncogenic virus
Viral gene
Peptide from a protein encoded by an oncogenic virus; T cell response

FIGURE 18.2 Tumor neoantigens. **A,** Normal cells display self peptides on MHC molecules, but there is no T cell response due to mechanisms of self-tolerance. **B,** Tumor neoantigens are most often encoded by somatic mutations, which produce mutated peptides that have new T cell receptor *(TCR)* contact residues and are recognized as foreign by the patient's T cells. **C,** Tumors caused by oncogenic viruses produce viral proteins that stimulate $CD8^+$ T cells specific for the infected cancer cells. *MHC,* Major histocompatibility complex.

Ineffective adaptive immune responses to cancers can be overcome by therapeutic strategies that stimulate such responses, such that antitumor T cells can be activated to effectively kill tumor cells. As we will discuss later in this chapter, this realization has spurred new directions in cancer immunotherapy in which augmentation of the host antitumor response is the goal of treatment.

The existence of specific antitumor immunity implies that tumors must express antigens that are recognized as foreign by the host. The nature and significance of these antigens are described next.

Tumor Antigens

Immune responses to malignant tumors are targeted to various types of molecules that cancer cells express and may be recognized by the immune system. Protein antigens that stimulate T cell responses are the most important types of antigens for protective antitumor immunity. In the past, the term tumor antigen has been used to encompass many different molecules expressed by tumor cells that are detected by the binding of antitumor antibodies, whether or not they stimulate protective immune responses. The tumor antigens that elicit T cell immune responses can be classified into several groups.

Neoantigens: Antigens Encoded by Mutated Genes

Tumor neoantigens are proteins encoded by mutated genes, which appear foreign to the immune system because they do not exist in normal cells and newly arise as a cancer develops. Usually, these neoantigens are encoded by genes carrying passenger mutations, which are point mutations or deletions that are unrelated to the development or malignant phenotype of the tumors (Fig. 18.2B). The occurrence of passenger mutations reflects the genomic instability of cancer cells. Alternatively, a smaller number of neoantigens may be encoded by driver mutations in tumor-promoting oncogenes or tumor suppressor genes. Because T cells recognize only peptides bound to major histocompatibility complex (MHC) molecules, tumor neoantigens can be recognized only if peptides carrying the mutated amino acid sequences can bind to the patient’s MHC alleles. Exome sequencing of many cancers has revealed large numbers of passenger mutations, and computer algorithms have been used to predict which of these mutations occur within peptide sequences that are likely to bind to the cancer patient's MHC alleles. Studies of cancer patients' T cells indicate that the tumor neoantigen peptides predicted to bind to MHC molecules in individual patients do, in fact, stimulate T cell responses in those patients, and that the number of different tumor antigen–specific T cell clones that are activated correlates with the number of mutations in the cancer. An example of this is a subset of cancer patients whose tumors have mutations in genes encoding DNA mismatch repair proteins, which result in a very high mutational burden. These patients have strong T cell responses to their tumors and are most likely to benefit from therapies designed to activate T cells, discussed later in the chapter.

Antigens of Oncogenic Viruses

The products of oncogenic viruses function as tumor antigens and elicit specific T cell responses that may serve to eradicate virus-induced tumors. Viruses are implicated in the development of a variety of tumors in humans and experimental animals. Examples in humans include the Epstein-Barr virus (EBV), which is associated with B cell lymphomas and nasopharyngeal carcinoma, and human papillomavirus (HPV), which is associated with carcinomas of the uterine cervix, oropharynx, and other sites. In most of these DNA virus–induced tumors, viral DNA integrates into host DNA and virus-encoded protein antigens are found in the nucleus, cytoplasm, or plasma membrane of the tumor cells (Fig. 18.2C). These endogenously synthesized viral proteins can be processed and presented by MHC molecules on the tumor cell surface. Some viruses, such as hepatitis B and C, are associated with cancer but are not directly oncogenic. It is thought they promote tumors by inducing chronic inflammatory reactions in which tumor-promoting growth factors and other signals are generated. The tumor cells may contain viral antigens, but this is highly variable.

The ability of adaptive immunity to prevent the growth of DNA virus–induced tumors has been established by many observations.

For instance, EBV-associated lymphomas and HPV-associated cervical cancers arise more frequently in immunosuppressed individuals, such as allograft recipients receiving immunosuppressive therapy and patients with AIDS. The efficacy of virus-specific adaptive immunity to prevent tumors may be due in large part to preventing infection and eliminating infected cells before cancers develop. Vaccination to prevent infection by these viruses also decreases the incidence of virus-associated cancers. A vaccine against HPV has reduced the incidence of cervical cancer and other HPV-associated lesions. The vaccine is composed of recombinant HPV capsid proteins from the most common oncogenic strains of HPV, which form virus-like particles free of viral genome. Vaccination against hepatitis B virus has reduced the incidence of HBV-associated liver cancer.

Overexpressed Cellular Proteins

Some tumor antigens are the products of genes that are silenced in normal cells and derepressed in tumor cells or are proteins made by normal cells but produced in excessive amounts by tumors. These antigens are not inherently foreign for the host, but nevertheless they stimulate immune responses. There are several possible explanations for their immunogenicity. Normally, the antigens may be expressed for a limited time or at a particular location—for example, only during embryonic development or only in tissues that are not accessible to the immune system—so there is no long-lived immunologic tolerance to these proteins. Expression in a tumor later in life or in locations that are not protected from immune cells may be enough to stimulate immune responses. The amount of antigen produced in a patient with cancer may be abnormally high, because of overexpression in each tumor cell or an abundance of tumor cells, and this too may be enough to elicit an active immune response.

Major categories of unmutated tumor antigens that are more abundant in tumors than in normal tissues include cancer-testis antigens, proteins encoded by amplified genes, and tissue differentiation antigens (Fig. 18.3). The expression of only some of these structurally unaltered tumor antigens is sufficiently different from expression in normal cells to stimulate protective immunity in patients. However, many of these tumor antigens are targets for antibody therapy and potential candidates for tumor vaccines.

- ***Cancer-testis antigens are proteins expressed in gametes and trophoblasts and in many types of cancers but not in normal somatic tissues*** (Fig. 18.3A) **.** The first cancer-testis antigens identified were melanoma-associated antigens (MAGEs). They are expressed in melanomas and many other types of tumors and in normal testis. Subsequently, several other unrelated gene families have been identified that encode antigens expressed by melanoma cells and are recognized by CTL clones derived from melanoma patients. The MAGE proteins and these other melanoma antigens are silent in most normal tissues, except the testis and placental trophoblast, but they are expressed in a variety of malignant tumors. More than 200 cancer-testis genes in over 40 different gene families have been identified. About half are encoded by genes on the X chromosome, and the rest are distributed on the other chromosomes. It has been postulated that in most somatic cells, the genes encoding these proteins are silenced by epigenetic mechanisms such as methylation of the promoter regions, but the loci are demethylated in cancer cells, allowing the genes to be expressed.
- ***Some proteins are expressed at abnormally high levels in tumor cells because the genes encoding these proteins are amplified*** (Fig. 18.3B) **.** One example of such a protein is the oncogenic epidermal growth factor variant called HER2/NEU, which is overexpressed in some breast cancers. There is no evidence that this protein elicits protective immune responses in patients, presumably because it is present in normal cells and induces tolerance. A

monoclonal antibody targeting HER2 is used to treat patients whose tumors show high HER2 expression.

- ***Differentiation antigens are found on tumor cells and on the cell types of origin of the tumors but not on cells from other tissues*** (Fig. 18.3C) **.** Two examples of such differentiation antigens in melanomas are tyrosinase, an enzyme involved in melanin biosynthesis, and MART-1 (Melan A), a protein required for melanosome function. Both $CD8^+$ CTLs and $CD4^+$ helper T cell responses specific for tyrosinase and MART-1 peptides are found in patients with melanoma, perhaps because these antigens are expressed at high levels due to the large number of tumor cells. However, in many cases, differentiation antigens do not induce immune responses because they are normal self antigens. Even in these situations, differentiation antigens are important in oncology because they aid in accurate diagnosis of tumor types and serve as targets for passive immunotherapy. For example, some lymphomas and leukemias arise from B cells and express surface markers characteristic of this lineage, such as CD19 and CD20. Antibody and T cell therapies targeted against these proteins are used to treat the cancers.

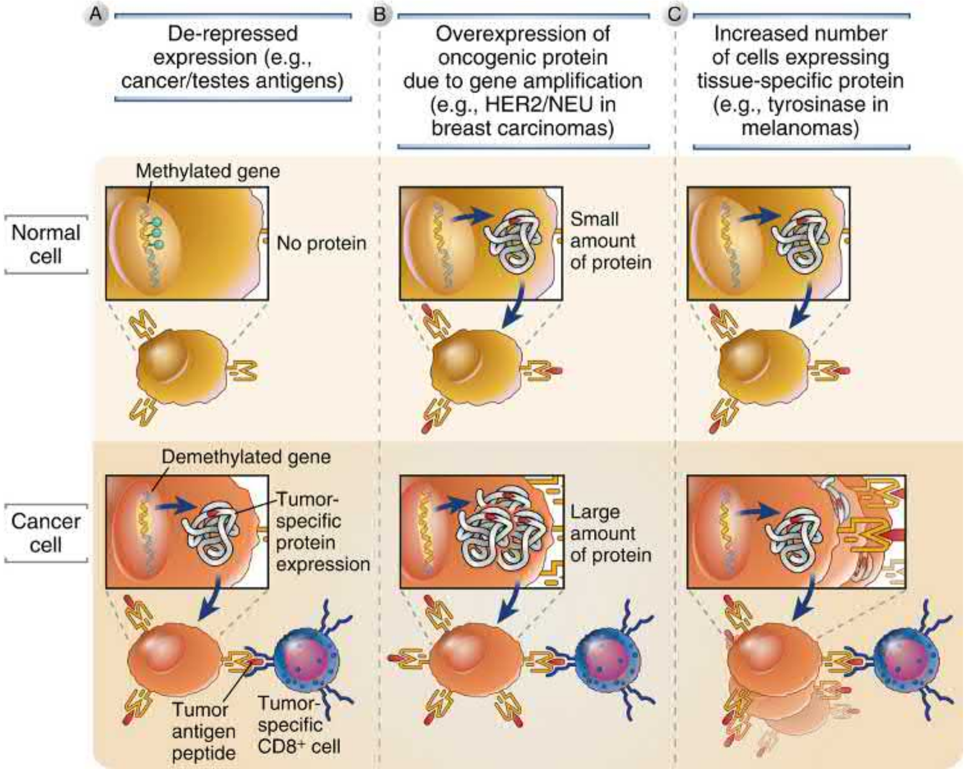

A
De-repressed expression (e.g., cancer/testes antigens)
B
Overexpression of oncogenic protein due to gene amplification (e.g., HER2/NEU in breast carcinomas)
C
Increased number of cells expressing tissue-specific protein (e.g., tyrosinase in melanomas)
Normal cell
Methylated gene
No protein
Small amount of protein
Cancer cell
Demethylated gene
Tumor-specific protein expression
Large amount of protein
Tumor antigen peptide
Tumor-specific CD8+ cell

FIGURE 18.3 Unmutated tumor antigens.Proteins that are not mutated but are expressed more abundantly by tumors than normal cells may induce T cell response in their hosts. Many of these tumor antigens include proteins, such as cancer-testis antigens, encoded by genes that are normally not expressed in most cells of adults, because of epigenetic suppression, but are depressed in tumor cells **(A).** Some tumor antigens may be overexpressed because of gene amplification, such as the HER2/NEU protein, which is abundant in many breast carcinomas **(B).** Tissue-specific antigens are proteins expressed by both cancer cells and the normal cell types from which tumors are derived, such as tyrosinase made by both melanocytes and malignant melanoma cells. Because of either gene deregulation or the

abundance of the tumor cells, the amount of these proteins is high in the tumors, leading to T cell responses **(C).**

Other Antigens of Tumors

Many attempts have been made to detect antigens in tumor cells and in the plasma of patients with cancer by producing antibodies against tumors and using these as screening reagents. Several classes of tumor antigens have been identified by this approach. It is, however, now clear that most of these antigens are produced even in normal cells, especially under conditions of tissue injury and inflammation. Therefore, the role of these antigens in tumor immunity is uncertain.

Oncofetal Antigens

Oncofetal antigens were the name given to proteins thought to be expressed at high levels in cancer cells and in fetal but not adult tissues. However, their expression in adults is not limited to tumors, but is increased in tissues and in the circulation in various inflammatory conditions, and the antigens are found in small quantities even in normal adult tissues. There is also no evidence that oncofetal antigens are inducers of antitumor immunity. Thus, their usefulness as tumor markers, targets of antibodies, or vaccine candidates is limited. The two most studied oncofetal antigens are carcinoembryonic antigen (CEA) and α-fetoprotein (AFP).

CEA (CD66) is a highly glycosylated membrane protein that functions as an intercellular adhesion molecule. High CEA expression is normally restricted to cells in the gut, pancreas, and liver during the first two trimesters of gestation. Its expression is increased in many carcinomas of the colon, pancreas, stomach, and breast, and serum levels are also increased in these patients. Serum CEA can, however, be elevated in the setting of nonneoplastic diseases, such as chronic inflammatory conditions of the intestines or liver, so it is of limited clinical utility.

AFP is a circulating glycoprotein normally synthesized and secreted by the yolk sac and liver in fetal life. Fetal serum concentrations can be as high as 2 to 3 mg/mL, but serum concentrations in adults are low. Serum levels of AFP can be elevated in patients with hepatocellular carcinoma, germ cell tumors, and occasionally gastric and pancreatic cancers. An elevated serum AFP level is sometimes used as an indicator of advanced liver or germ cell tumors or of recurrence of these tumors after treatment.

Altered Glycolipid and Glycoprotein Antigens

Most human and experimental tumors express higher than normal levels or abnormal forms of surface glycoproteins and glycolipids, including gangliosides, blood group antigens, and mucins. Tumors often have dysregulated expression of the enzymes that synthesize the carbohydrate side chains of mucins, which leads to the appearance of tumor-specific epitopes on the carbohydrate side chains or on the abnormally exposed polypeptide core. Several mucins have been the focus of diagnostic and therapeutic studies. One of these, a mucin called MUC1, is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium, a site that is relatively sequestered from the immune system. In some carcinomas, however, MUC1 is expressed in a nonpolarized fashion and contains new, tumor-specific carbohydrate and peptide epitopes detectable by mouse monoclonal antibodies. Whether effective vaccines can be developed with these epitopes remains an open question.

Immune Responses To Tumors

Both innate and adaptive immune responses to tumors can be detected in patients and experimental animals, and various immune mechanisms can kill tumor cells in vitro. The challenge for tumor immunologists has been to determine which of these mechanisms may contribute significantly to protection against tumors and to develop therapies that enhance these effector mechanisms in ways that are tumor specific. Recent technical advances in characterizing tumor antigen–specific immune responses and data from studies of cancer patients treated with drugs that stimulate T cells have indicated that CTLs are the most important contributors to host immune defense against tumors. In this section, we will review the evidence for antitumor immunity mediated by T cells and other immune effector mechanisms.

T Lymphocytes

The principal mechanism of immune protection against tumors is killing of tumor cells by CD8$^+$ CTLs (Fig. 18.4) **.** The ability of CTLs to provide effective antitumor immunity in vivo is clearly seen in animal experiments using carcinogen-induced and DNA virus–induced tumors. CTLs may perform a surveillance function by recognizing and killing potentially malignant cells that express peptides that are derived from tumor antigens and are presented in association with class I MHC molecules. Tumor-specific CTLs can be isolated from animals and humans with established tumors, and, as discussed earlier, there is evidence that the prognosis of human tumors, including common types such as colon carcinomas, is more favorable when more CTLs are present within the tumor (see Fig. 18.1D). Furthermore, mononuclear cells derived from the inflammatory infiltrate in human solid tumors, called tumor-infiltrating lymphocytes (TILs), contain CTLs with the capacity to kill the tumor from which they were isolated. Importantly, the inability to detect functional tumor-specific CTLs in some patients may be because of regulatory mechanisms exploited by the tumor to suppress CTL responses, and new therapies that block these regulatory mechanisms lead to the development of strong CTL responses against the tumor (discussed later).

CD8$^+$ T cell responses specific for tumor antigens may require cross-presentation of the tumor antigens by dendritic cells (DCs). Most tumor cells are not derived from antigen-presenting cells (APCs) and therefore are not present in secondary lymphoid organs where they can display antigens to naive T cells, nor do the tumor cells express costimulatory molecules needed for naive T cell activation. Thus, to initiate antitumor CD8$^+$ T cell responses, tumor antigens have to be presented by DCs, the best APCs for transporting tumor antigens to secondary lymphoid organs and for activating naive T cells. DCs at the site of a tumor can ingest tumor cells or their protein antigens, carry the tumor antigens to lymph nodes, and colocalize with naive CD8$^+$ T cells (see Chapter 6). Furthermore, the DCs can deliver the ingested proteins from phagosomes into the cytosol so that they are processed by proteasomes into peptides that are then displayed bound to class I MHC molecules for recognition by CD8$^+$ T cells (Fig. 18.5). This process of cross-presentation, or cross-priming, is mainly carried out by the cDC1 subset of DCs, as described in earlier chapters in the context of initiating CD8$^+$ responses to viruses. DCs also express costimulators, and these or helper T cells that are activated at the same time provide the signals needed for differentiation of naive CD8$^+$ T cells into tumor-specific CTLs. Once effector CTLs are generated, they are able to recognize and kill the tumor cells without a requirement for costimulation.

CD4$^+$ helper T cells contribute to antitumor immune responses by several mechanisms. CD4$^+$ T cell responses to tumor antigens are commonly found in animal models and patients with cancer, and the presence of Th1 cells, like CTLs, in human tumors correlates with good prognosis. Some studies show a therapeutic benefit of adoptive transfer of tumor antigen–specific CD4$^+$ T cells into the host. The antitumor effects of Th1 cells may reflect their known role in enhancing CD8$^+$ T cell responses (see Chapter 11) and activating macrophages, through the secretion of interferon-γ (IFN-γ) (see Chapter 10). IFN-γ can increase tumor cell class I MHC expression and sensitivity to lysis by CTLs. The importance of IFN-γ in tumor immunity is demonstrated by the finding of increased incidence of tumors in knockout mice lacking this cytokine, its receptor, or IFN-γ induced signaling molecules.

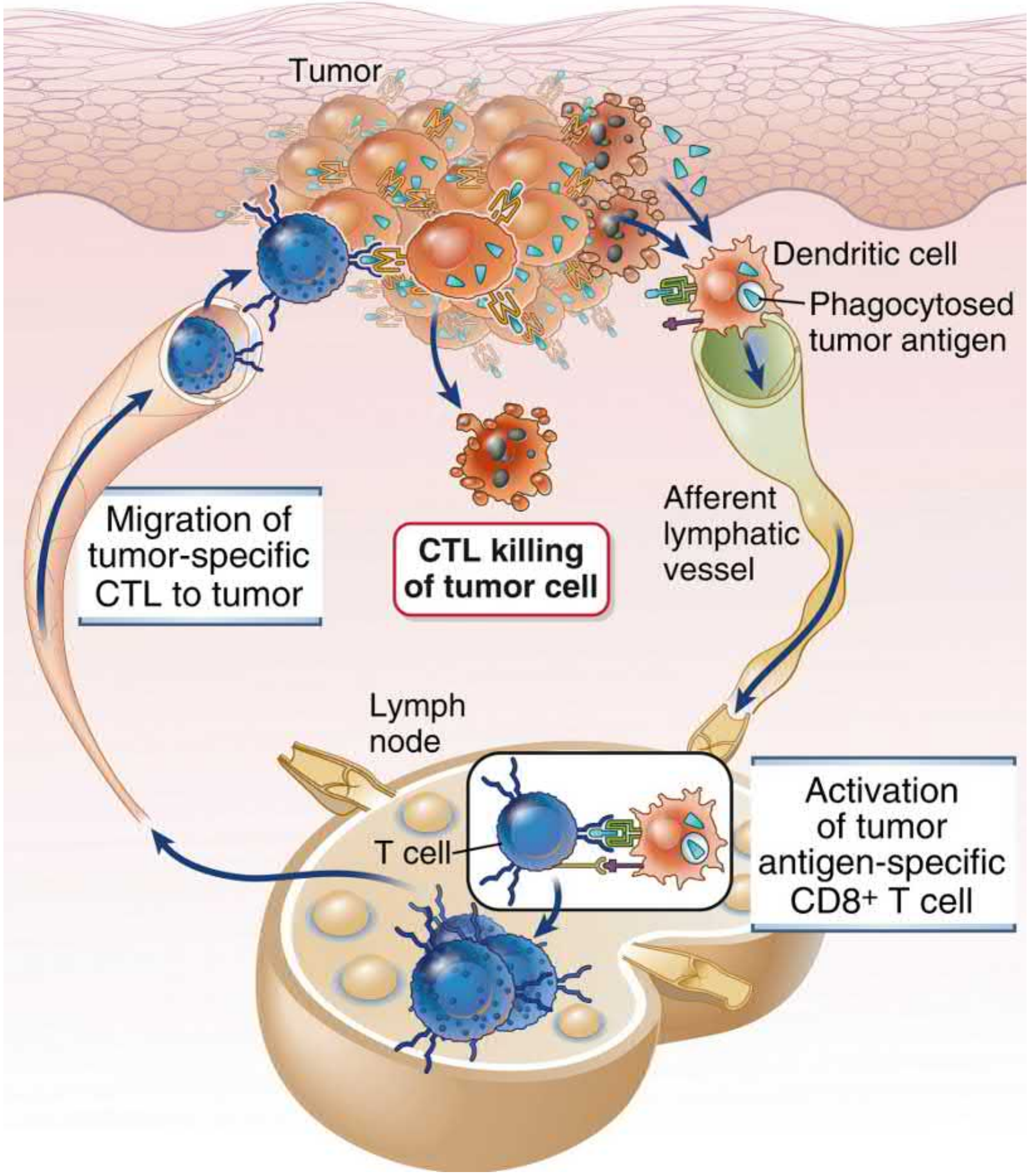

Tumor
Dendritic cell
Phagocytosed tumor antigen
Migration of tumor-specific CTL to tumor
CTL killing of tumor cell
Afferent lymphatic vessel
Lymph node
T cell
Activation of tumor antigen-specific CD8+ T cell

FIGURE 18.4 Cytotoxic T lymphocyte response against tumors. Tumor antigens are picked up by host dendritic cells, and responses are initiated in secondary lymphoid organs. Tumor-specific cytotoxic T lymphocytes *(CTLs)* migrate back to the tumor and kill tumor cells. CD4$^+$ T cell responses against tumors involve similar initiating steps to generate tumor-specific helper T cells, but the antitumor effector mechanisms are

different. Other mechanisms of tumor immunity are not shown.

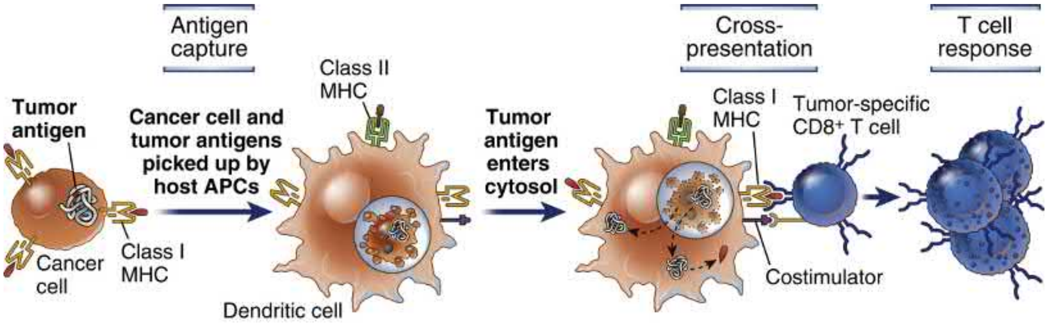

Antigen capture
Cross-presentation
T cell response
Tumor antigen
Cancer cell and tumor antigens picked up by host APCs
Class II MHC
Tumor antigen enters cytosol
Class I MHC
Tumor-specific CD8+ T cell
Cancer cell
Class I MHC
Dendritic cell
Costimulator

FIGURE 18.5 Activation of tumor-specific CD8$^+$ T cells by cross-presentation.Protein tumor antigens, or the cancer cells that produce the antigen, are ingested by dendritic cells *(DCs)* into endocytic vesicles. The antigens are then delivered into the cytosol, where they enter the class I major histocompatibility complex *(MHC)* antigen processing and presentation pathway, resulting in the display of tumor peptides bound to class I MHC displayed on the DC surface, along with costimulatory molecules. Naive CD8$^+$ T cells specific for these peptide-MHC antigens can then be activated. *APCs,* Antigen-presenting cells; *CTL,* cytotoxic T lymphocyte.

The demonstration that the numbers of different types of T cells within resected tumors correlates with the likelihood of metastatic disease has led to the idea that determining an immune score for cancers may be useful to assess prognosis and direct treatment options. This has been most thoroughly studied in some medical centers in cases of colon cancer, in which a score was given to tumors based on the number of CD45RO$^+$ memory T cells and CD8$^+$ CTLs in the margins of resected tumors. A low score was found to predict a higher chance for relapse, metastases, and death within 5 years compared with tumors with a high score, even when comparing tumors with no evidence of lymph node or distant metastases at the time of resection. In some studies, the immune score was found to have greater prognostic value than the histologic evaluation of the tumor. Current research is focused on expanding the use of immune scores for a wider range of tumors and broadening the analyses of resected tumors to include more subsets of immune cells by immunohistochemistry and other methods. Additional immune/inflammatory gene expression patterns of individual tumors are also being studied and may supplement immune scores.

Antibodies

Tumor-bearing hosts often produce antibodies against various tumor antigens, but the significance of these antibodies in protecting against cancers is unknown. Antibodies may kill tumor cells by activating complement or by antibody-dependent cell-mediated cytotoxicity, in which Fc receptor–bearing macrophages or natural killer (NK) cells mediate the killing. However, there is little evidence that humoral immune responses against tumors have a significant effect in preventing the development or progression of tumors. There are several approved and effective antitumor antibodies that are used to provide passive immunity against tumors, discussed later.

Natural Killer Cells

NK cells are capable of killing many types of tumor cells and may contribute to immune surveillance against cancers. Some studies have indicated that people with defects in NK cell function or numbers caused by genetic mutations or with lower than normal NK cell activity without known genetic defects are at higher risk than the general population for developing certain types of virally caused tumors. Mouse studies also have shown that genetic defects in NK cell function or depletion of NK cells by antibodies enhances tumor growth and metastases. Although these findings support a contribution of NK cells to immune surveillance, these cells usually represent only a small fraction of the inflammatory infiltrates present in most human and mouse tumors, and their role in immune eradication of established tumors is not clear.

Tumor cells become susceptible to killing by NK cells when they downregulate expression of class I MHC or they upregulate expression of ligands that bind activating NK cell receptors. NK cells express inhibitory receptors that bind class I MHC molecules expressed on healthy cells (see Chapter 4). As we will see later, some tumors lose expression of class I MHC molecules, as a result of selection against class I MHC–expressing cells that are readily killed by CTLs. This loss of class I MHC molecules makes the tumors particularly good targets for NK cells. In addition, many tumors express ligands for the NKG2D activating receptor on NK cells, such as MIC-A, MIC-B, and ULB, and NKG2D signaling can override inhibitory signals from class I MHC binding receptors. NK cells also may be activated to kill tumor cells coated with antitumor antibodies by antibody-dependent cell-mediated cytotoxicity. The tumoricidal capacity of NK cells is increased by cytokines, including interleukin-2 (IL-2), IL-15, and IL-12, and the antitumor effects of these cytokines in vivo are partly attributable to stimulation of NK cell activity.

Macrophages

Macrophages are capable of both inhibiting and promoting the growth and spread of cancers, depending on their activation state. Classically activated M1 macrophages, discussed in Chapter 10, can kill many types of tumor cells. How macrophages are activated by tumors is not known. A possible mechanism is recognition of damage-associated molecular patterns from dying tumor cells by macrophage innate immune receptors. Macrophages in tumors also may be activated to kill tumor cells by IFN-γ produced by tumor-specific Th1 cells, CTLs, and NK cells. This may be why a large number of Th1 cells in some tumors is correlated with a good prognosis. M1 macrophages can kill tumor cells by mechanisms that they also use to kill infectious organisms, including the liberation of lysosomal enzymes, nitric oxide, and reactive oxygen species. We will discuss how M2 macrophages promote tumor growth in the next section.

The Role of Innate and Adaptive Immunity in Promoting Tumor Growth

Although much of the emphasis in tumor immunology has been on the role of the immune system in eradicating tumors, it is clear that the immune system may also contribute to the growth of some solid tumors. In fact, chronic inflammation has long been recognized as a risk factor for development of tumors in many different tissues, especially those affected by chronic inflammatory diseases such as Barrett's esophagus and ulcerative colitis. Some cancers associated with infections are also considered to be an indirect result of the tumor-promoting effects of the chronic inflammatory states that are induced by the infectious organisms. These include gastric carcinoma and lymphoma in the setting of chronic *Helicobacter pylori* infection and hepatocellular carcinomas associated with chronic hepatitis B and C virus infections. Although the mechanisms by which chronic inflammation can promote tumor development are not well understood, there are several possibilities supported by data in rodent models.

Myeloid cells of the innate immune system are considered the most direct tumor-promoting culprits among immune cells. They may contribute to malignant transformation of cells by generating free radicals that cause DNA damage and lead to mutations in tumor suppressor genes and oncogenes. Some data suggest that cells of the innate immune system, including mast cells, neutrophils, and macrophages, secrete soluble factors that promote cell cycle progression and survival of tumor cells. The transcription factor NF-κB (nuclear factor κB), which is a key mediator of innate immune responses, may play an important role in inflammation-associated cancer progression. Tumor-associated macrophages of the alternatively activated (M2) phenotype, as well as other cells, are sources of vascular endothelial growth factor (VEGF), a growth factor that promotes angiogenesis, and matrix metalloproteinases, enzymes that modify the extracellular tissue (Fig. 18.6). Therefore, chronic activation of some innate immune cells is characterized by angiogenesis and tissue remodeling, which favor tumor growth and spread.

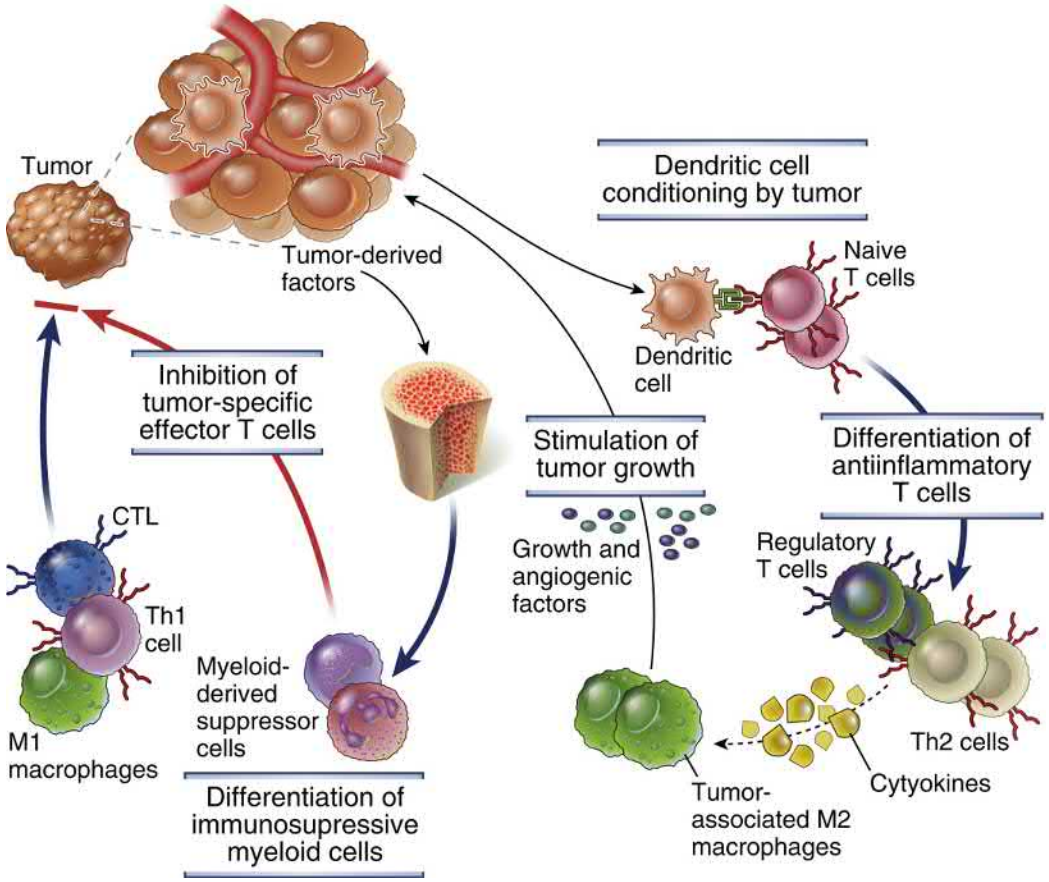

Tumor
Tumor-derived factors
Dendritic cell conditioning by tumor
Naive T cells
Dendritic cell
Inhibition of tumor-specific effector T cells
Stimulation of tumor growth
Differentiation of antiinflammatory T cells
CTL
Growth and angiogenic factors
Regulatory T cells
Th1 cell
Myeloid-derived suppressor cells
M1 macrophages
Th2 cells
Cytyokines
Tumor-associated M2 macrophages
Differentiation of immunosupressive myeloid cells

FIGURE 18.6 Promotion of tumor growth by the antiinflammatory tumor microenvironment. Although inflammation can promote malignant transformation of cells and the development of cancers, established tumors often create a microenvironment that suppresses antitumor immunity and promotes cancer cell growth. Tumors alter the phenotype of dendritic cells in ways that promote the differentiation of antiinflammatory regulatory T cells and Th2 cells, which in turn promote differentiation and accumulation of M2 macrophages and myeloid-derived suppressor cells. These cells block the action of antitumor cytotoxic T lymphocytes and Th1 cells and provide growth factors for tumor cells and tumor blood vessels. (The *blue arrows* show changes or movement of cells, the *black*

arrows show effects of released factors from tumors on immune cells or vice versa, and the *red arrow* refers to inhibition.)

Alternatively activated macrophages and less well-characterized cell populations, such as myeloid-derived suppressor cells (MDSCs), may also promote tumor growth indirectly by inhibiting effective antitumor immunity. The role of these suppressor cells in immune evasion is discussed later.

The adaptive immune system can enhance tumor development in several ways. In response to tumors, DCs may be conditioned to drive CD4$^+$ T differentiation to Th2 cells or regulatory T cells (Tregs), both of which suppress Th1 and CTL responses that destroy tumors and increase the development of M2 macrophages and other cell types that promote tumor growth (see Fig. 18.6). Experimental evidence in mice suggests that B lymphocytes may contribute to tumor progression, but this remains controversial because B cells also have been linked to tumor regression.

The tumor-promoting effects of the immune system are paradoxical and a topic of active investigation at present. A major problem is that the nature of the tumor-promoting immune stimuli varies among different tumors, and a cell type that promotes some tumors may inhibit others. As a result, it has been difficult to definitively establish the most important tumor-promoting mechanisms. These effects of chronic inflammation are theoretically targets for pharmacologic intervention because there is a large variety of effective antiinflammatory drugs already available. The challenge for oncologists is to achieve a beneficial balance in which protective antitumor immune responses are not compromised while potentially harmful tumor-promoting inflammatory reactions are controlled.

Evasion of Immune Responses by Tumors

To grow in immunocompetent hosts, tumors have to evade or resist host immune responses. Several mechanisms of immune evasion by tumors have been hypothesized and supported by experimental evidence or by clinical success of therapeutic approaches that target evasion mechanisms (Fig. 18.7). A major focus of tumor immunology is to understand these immune evasion mechanisms, with the goal that interventions to prevent immune evasion will increase the immunogenicity of tumors and maximize the effectiveness of the host response. Most evasion mechanisms can be categorized as either active inhibition of antitumor immune responses or loss of antigens that drive these responses.

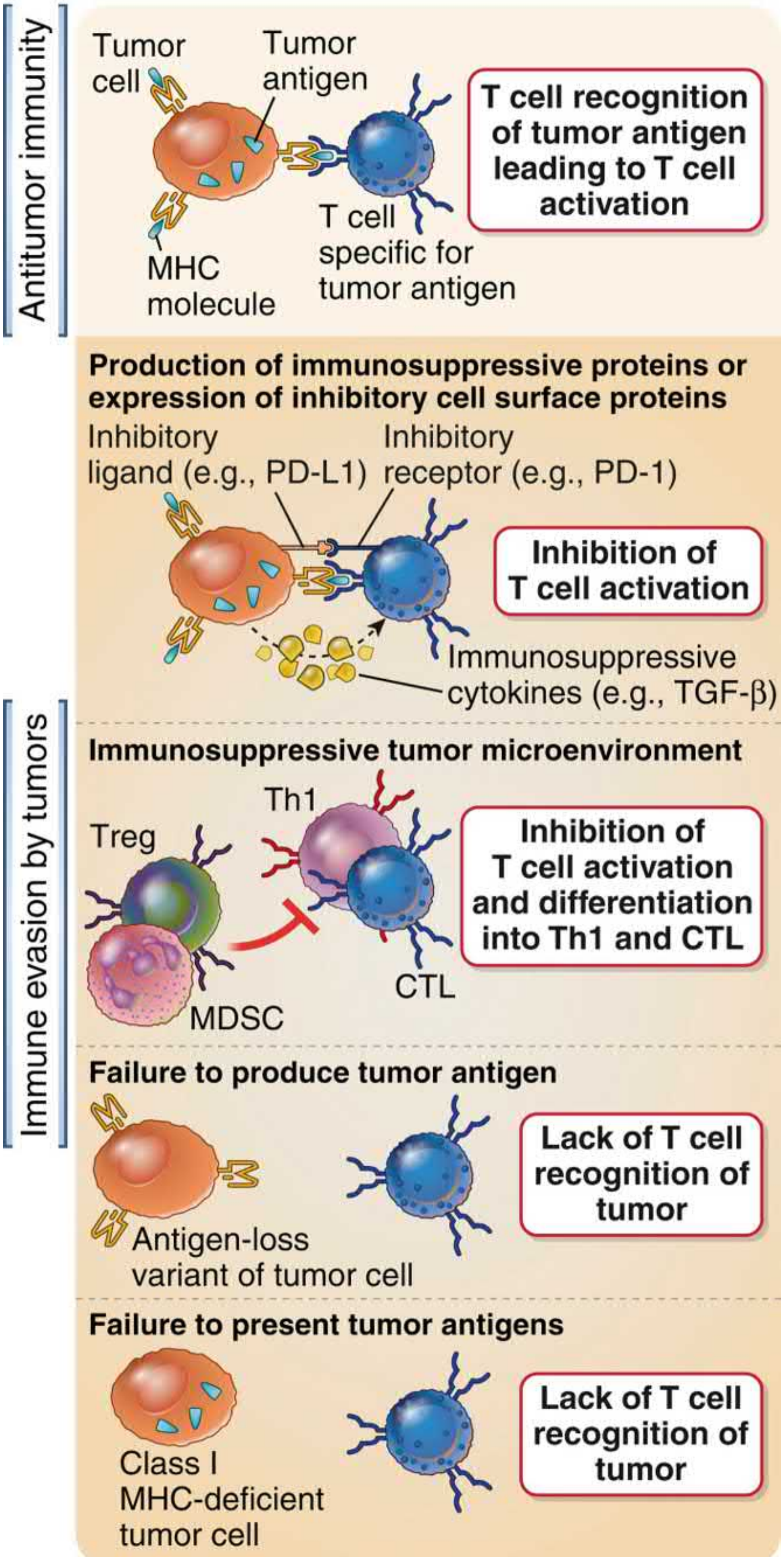

Antitumor immunity
Tumor cell
Tumor antigen
MHC molecule
T cell specific for tumor antigen
T cell recognition of tumor antigen leading to T cell activation
Immune evasion by tumors
Production of immunosuppressive proteins or expression of inhibitory cell surface proteins
Inhibitory ligand (e.g., PD-L1)
Inhibitory receptor (e.g., PD-1)
Inhibition of T cell activation
Immunosuppressive cytokines (e.g., TGF-β)
Immunosuppressive tumor microenvironment
Th1
Treg
MDSC
CTL
Inhibition of T cell activation and differentiation into Th1 and CTL
Failure to produce tumor antigen
Antigen-loss variant of tumor cell
Lack of T cell recognition of tumor
Failure to present tumor antigens
Class I MHC-deficient tumor cell
Lack of T cell recognition of tumor

FIGURE 18.7 Mechanisms by which tumors escape immune defenses.Antitumor immunity develops when T cells recognize tumor antigens and are activated. Tumor cells may evade immune responses by losing expression of antigens or major histocompatibility complex *(MHC)* molecules, by producing ligands for T cell inhibitory receptors and immunosuppressive cytokines. The tumor micromovement, which includes immunosuppressive myeloid cells and lymphocytes, inhibits the activation, differentiation, and infiltration of antitumor effector T cells. *CTL,* Cytotoxic T lymphocyte; *MDSC,* myeloid-derived supressor cell; *PD-1,* programmed cell death protein-1; *PD-L1,* PD-ligand 1; *TGF-1β,* transforming growth factor-1; *Treg,* regulatory T cell;

Molecules and Cells That Inhibit Immune Responses to Tumors

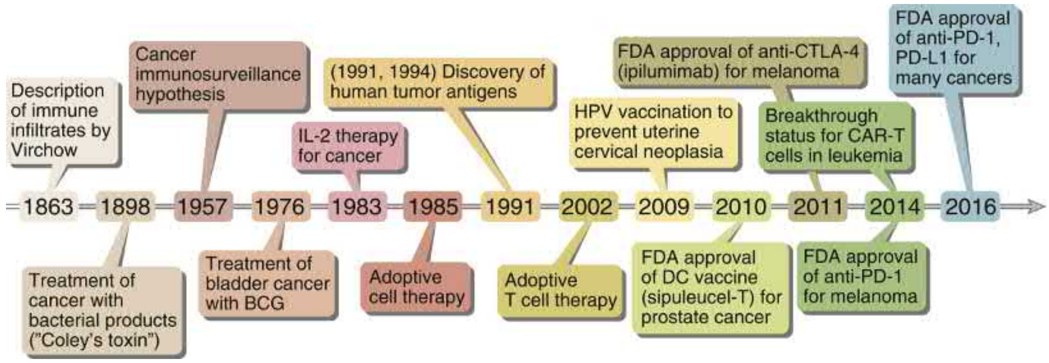
Tumors evade antitumor T cell responses by engaging T cell inhibitory (immune checkpoint) molecules that normally function to prevent autoimmunity or regulate immune responses to microbes. This has been shown most clearly for CTLA-4 (cytotoxic T lymphocyte–associated protein 4) and PD-1 (programmed cell death protein-1), two of the best-defined inhibitory pathways in T cells (see Chapter 15). The importance of these molecules in tumor immune evasion became clear from experimental work in mice showing that blockade of these inhibitors enhanced anti-tumor immunity, followed by successful clinical trials and now widely adopted use of anti-PD-1 and anti-CTLA-4 antibodies to treat cancers. Tumors are able to resist T cell immune attack by upregulating the expression of PD-1 and CTLA-4 on tumor-specific

T cells, probably as a result of chronic T cell stimulation by persistent tumor antigens. Tumor-infiltrating T cells often have a dysfunctional (exhausted) phenotype that was first described in the context of chronic viral infections (see Chapter 11). Exhausted T cells are characterized by impaired effector functions and increased expression of CTLA-4, PD-1, and other inhibitory molecules. In addition to upregulating CTLA-4 on tumor-specific effector T cells, the tumor microenvironment may also promote differentiation of regulatory T cells, which express CTLA-4.

For PD-1 and CTLA-4 to function as T cell inhibitors, they must bind their ligands. The ligands for PD-1 are the B7 family proteins PD-L1 (PD-ligand 1) and PD-L2 (see Chapter 15). Many tumors express PD-L1 sometimes because of PDL1 gene amplification, and some tumors express PD-L2. IFN-γ produced by tumor-infiltrating T cells also induces PD-L1 expression on tumor cells and on myeloid cells in the tumor microenvironment. Thus, many tumors or cells surrounding tumors can engage PD-1 on infiltrating T cells and stop the T cells from performing their tumor-killing functions. The ligands for CTLA-4 are B7-1 and B7-2, which are costimulatory molecules mainly expressed on antigen-presenting cells in secondary lymphoid organs that work by engaging CD28. CTLA-4 binds B7 more strongly than CD28 does and thus acts as a competitive inhibitor of T cell costimulation (see Chapter 15). A possible reason why tumors can exploit CTLA-4 to regulate antitumor responses is that tumor antigens are often presented by APCs in the absence of strong innate immunity and thus with low levels of B7 costimulators on the APCs. These low levels may all be preferentially bound up by the high-affinity receptor CTLA-4 on effector T cells and Treg and therefore not accessible to CD28. In addition to PD-1 and CTLA-4, other inhibitory receptors expressed by tumor-specific T cells, including LAG3, TIM3, and TIGIT, also may contribute to inhibition of antitumor immune responses.

Secreted products of tumor cells may suppress antitumor immune responses. An example of an immunosuppressive tumor product is TGF-β, which is secreted by many tumors and inhibits the proliferation and effector functions of lymphocytes and macrophages (see Chapter 15).

Tregs may suppress T cell responses to tumors. Evidence from mouse tumor studies and patients with cancer indicates that Tregs can be found in the cellular infiltrates in certain tumors. Depletion of Tregs in tumor-bearing mice enhances antitumor immunity and reduces tumor growth. However, the role and prognostic value of Tregs present within human tumors remain uncertain and may vary among tumor types.



Description of immune infiltrates by Virchow
Cancer immunosurveillance hypothesis
IL-2 therapy for cancer
(1991, 1994) Discovery of human tumor antigens
HPV vaccination to prevent uterine cervical neoplasia
FDA approval of anti-CTLA-4 (ipilumimab) for melanoma
Breakthrough status for CAR-T cells in leukemia
FDA approval of anti-PD-1, PD-L1 for many cancers
1863
1898
1957
1976
1983
1985
1991
2002
2009
2010
2011
2014
2016
Treatment of cancer with bacterial products ("Coley's toxin")
Treatment of bladder cancer with BCG
Adoptive cell therapy
Adoptive T cell therapy
FDA approval of DC vaccine (sipuleucel-T) for prostate cancer
FDA approval of anti-PD-1 for melanoma

FIGURE 18.8 History of cancer immunotherapy.Some of the important discoveries in the field of cancer immunotherapy are summarized. *BCG,* Bacillus Calmette-Guérin; *CAR,* chimeric antigen receptor; *CTLA-4,* cytotoxic T lymphocyte–associated protein 4; *DC,* dendritic cell; *FDA,* U.S. Federal Drug Administration; *HPV,* human papillomavirus; *IL-2,* interleukin-2; *PD-1,* programmed cell death protein-1; *PD-L1,* PD-ligand 1.

Modified from Lesterhuis WJ, Haanen JB, Punt CJ. Cancer immunotherapy—revisited. *Nat Rev Drug Disc.* 2011;10:591.

Myeloid-derived suppressor cells (MDSCs) are immature myeloid precursors that accumulate in bone marrow, lymphoid tissues, blood, and tumors of tumor-bearing animals and cancer patients and suppress innate and T cell–mediated antitumor immune responses.

MDSCs are a heterogeneous collection of different myeloid cell types, including cells that are most similar to monocytes, neutrophils, or DCs. In addition to patients with tumors, MDSCs also accumulate in tissues of patients with chronic inflammatory diseases. MDSCs are reported to suppress innate and adaptive immune responses by many different mechanisms, including secretion of immunosuppressive cytokines, such as IL-10 and TGF-β; promotion of Treg differentiation; and inhibition of CTL and Th1 differentiation. Cyclooxygenase-2–dependent production of prostaglandin E2 may enhance the differentiation of MDSCs with these immunosuppressive functions. Although the presence of MDSCs in tumors correlates with impaired antitumor immune responses, there are many gaps in our knowledge about the nature of these cells, how they develop and function, and how they can be targeted for therapeutic purposes. As mentioned earlier, M2 macrophages activated by tumors may also inhibit antitumor immunity and promote tumor growth.

Loss of Tumor Antigen Expression

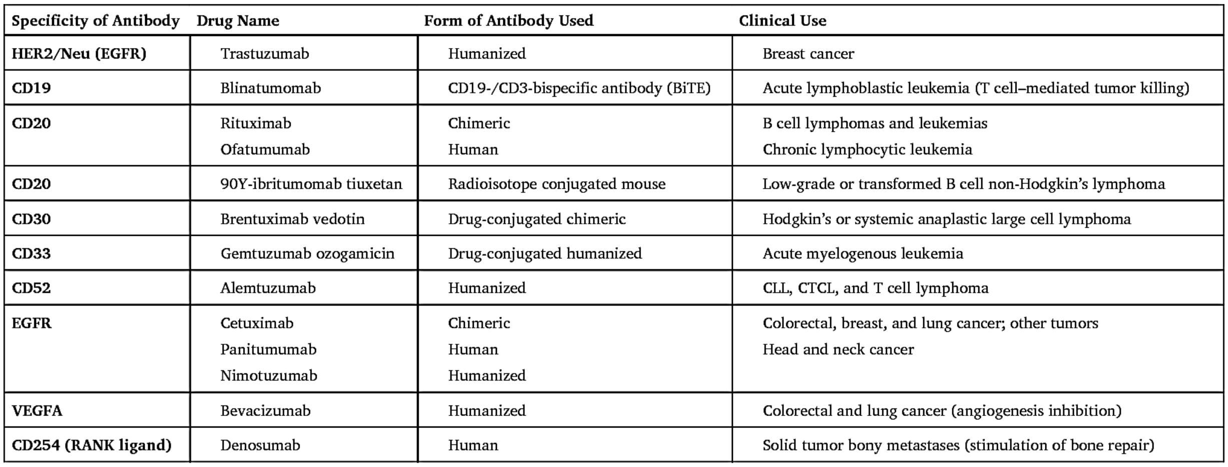
Immune responses to tumor cells impart selective pressures that result in the survival and outgrowth of variant tumor cells with reduced immunogenicity. Experiments comparing tumors that develop in normal mice versus Rag-deficient mice that lack adaptive immunity show that only in the setting of a normal immune system do tumors become less immunogenic over time, which is consistent with selective survival of less immunogenic clones. This phenomenon has been called immune editing, implying that the immune response selects for changes in tumors that help them evade the response. Given the high mitotic rate of tumor cells and their genetic instability, mutations or deletions in genes encoding tumor antigens are common. If these antigens are not required for growth of the tumors or maintenance of the transformed phenotype, the antigen-negative tumor cells will have a growth advantage in the face of the host immune response. Recent studies have confirmed this occurs in cancer patients. Tumor-specific antigens that drive T cell responses in the patients were identified by full exome sequencing and identification of mutant peptides that bound to the patients' MHC alleles. In those patients, tumor subclones could be detected that no longer carried the mutations that encode immunogenic neoantigens.

Class I MHC expression is often downregulated on tumor cells so they cannot present any tumor antigens to $CD8^+$ T cells. This is likely a more effective form of immune evasion than mutational loss of individual antigens. Various tumors show decreased synthesis of class I MHC molecules, or proteins required for class I MHC expression on the cell surface such as β2-microglobulin, or components of the antigen-processing machinery such as transporter associated with antigen processing-1 (TAP1) and TAP2 and subunits of the proteasome. Loss of expression of class I MHC molecules or of proteins involved in MHC assembly and antigen presentation are due to mutations in the genes encoding these molecules. Tumor cells with such mutations presumably have a selective survival advantage because they can evade CTL-mediated immune responses. As we discussed earlier, tumors that lose class I MHC are more likely to be recognized by NK cells. However, additional mutations that impair tumor cell expression of ligands for NK cell–activating receptors may emerge, promoting the outgrowth of subclones that also evade NK cell attack.

Immunotherapy For Tumors

Oncologists and immunologists have worked for many years on immunologic approaches to treat patients with cancer, but only recently have there been exciting and broadly applicable breakthroughs that have been successfully used to treat patients (Fig. 18.8). A major reason for interest in immunologic treatments is that most established therapies for cancer rely on drugs (chemotherapy) or radiation that kill dividing cells or block cell division, and these treatments have harmful effects on normal proliferating cells. As a result, the treatment of cancers causes significant morbidity and mortality. Immune responses to tumors can theoretically be highly specific for tumor cells and will not injure most normal cells. Therefore, immunotherapy has the potential for being the most tumor-specific treatment that can be devised. Recent advances in identifying tumor antigens and methods for genetically modifying T cells so they are specific for those antigens have brought us closer to tumor-specific immunotherapy. The breakthrough approaches now in practice that stimulate the immune response to control tumors are not entirely tumor antigen specific and do have side effects of damaging normal tissues. Nonetheless, these approaches provide great benefit to many patients.

Table 18.1



Specificity of Antibody	Drug Name	Form of Antibody Used	Clinical Use
HER2/Neu (EGFR)	Trastuzumab	Humanized	Breast cancer
CD19	Blinatumomab	CD19-/CD3-bispecific antibody (BiTE)	Acute lymphoblastic leukemia (T cell–mediated tumor killing)
CD20	Rituximab Ofatumumab	Chimeric Human	B cell lymphomas and leukemias Chronic lymphocytic leukemia
CD20	90Y-ibritumomab tiuxetan	Radioisotope conjugated mouse	Low-grade or transformed B cell non-Hodgkin's lymphoma
CD30	Brentuximab vedotin	Drug-conjugated chimeric	Hodgkin's or systemic anaplastic large cell lymphoma
CD33	Gemtuzumab ozogamicin	Drug-conjugated humanized	Acute myelogenous leukemia
CD52	Alemtuzumab	Humanized	CLL, CTCL, and T cell lymphoma
EGFR	Cetuximab Panitumumab Nimotuzumab	Chimeric Human Humanized	Colorectal, breast, and lung cancer; other tumors Head and neck cancer
VEGFA	Bevacizumab	Humanized	Colorectal and lung cancer (angiogenesis inhibition)
CD254 (RANK ligand)	Denosumab	Human	Solid tumor bony metastases (stimulation of bone repair)

Most of the antibodies listed bind to tumor antigens and destroy or inhibit the growth of the tumor cells. Antibodies that have antitumor effects by other mechanisms are indicated.

BiTE, Bispecific T cell engager; *CLL,* chronic lymphocytic leukemia; *CTCL,* cutaneous T-cell lymphoma; *EGFR,* epidermal growth factor receptor; *VEGEFA,* vascular endothelial growth factor A.

A second major reason to explore immunologic approaches for treating tumors is that cytotoxic drugs have been unsuccessful in achieving durable benefits in most cancers that have spread in the body beyond their site of origin. Because long-lasting memory is a cardinal feature of adaptive immune responses and immunity is systemic, it is possible that once an effective adaptive immune response to a tumor is initiated, it will be sustained for a long time and will be effective throughout the body. Because of this feature of the immune response, there is hope that some immunotherapeutic approaches will achieve long-term cures.

In this section, we describe the different modes of tumor immunotherapy that are currently used clinically or in development.

Passive Immunotherapy With Monoclonal Antibodies or Antibody-Like Molecules

Passive antibody therapy involves the transfer of tumor-specific antibodies into patients, which is a rapid and theoretically very specific approach but does not lead to long-lived immunity. Some monoclonal antibodies have been in use to treat cancers for over 20 years, and many more are now approved or in advanced development (Table 18.1). Furthermore, recombinant single polypeptide proteins with antibody-like antigen-binding sites, called single chain variable fragments, specific for tumor antigens have been developed for cancer treatment.

- Some antitumor antibodies bind to cell surface molecules on tumor cells and engage host effector mechanisms that kill the tumor cells (Fig. 18.9A). These mechanisms include NK cell–mediated cytotoxicity, complement-mediated lysis, and complement- or Fc receptor–mediated phagocytosis by macrophages (see Chapter 13). Several antitumor antibodies that are now approved for the treatment of certain cancers work in this way. For example, as mentioned earlier, anti-CD20 is used for treating B cell lymphomas, and it works by depleting all CD20-expressing cells, including B cells and B cell–derived lymphoma cells, mainly by antibody-dependent cellular cytotoxicity and perhaps also by complement activation.

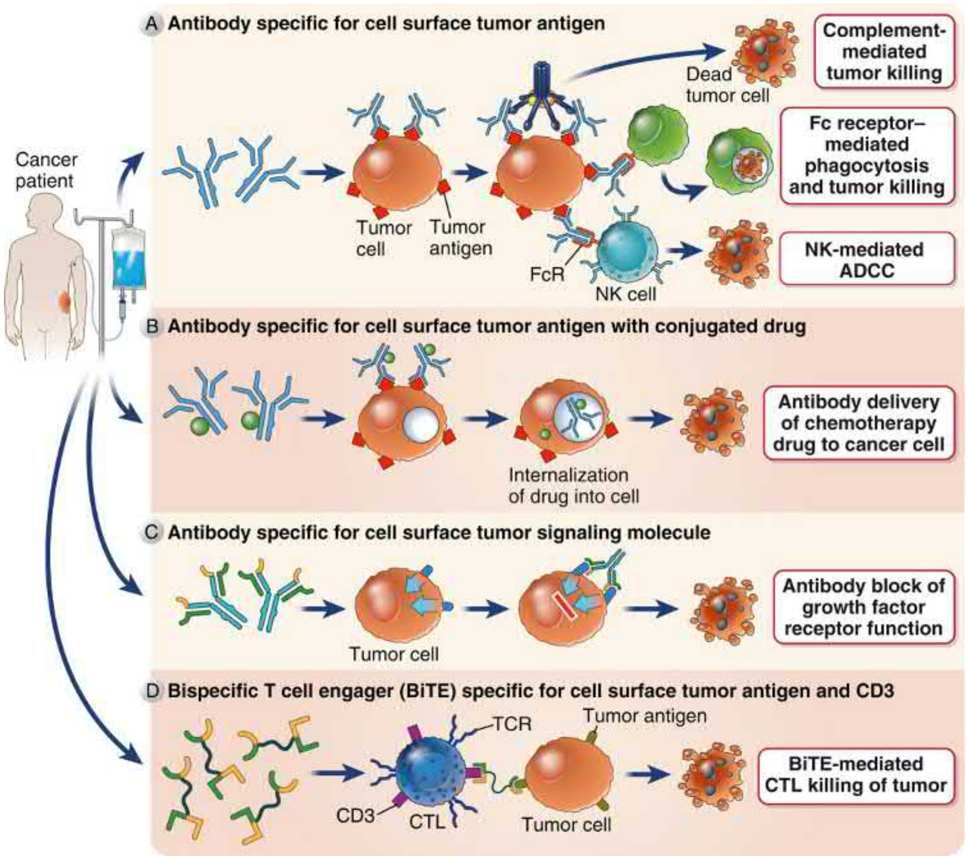

A Antibody specific for cell surface tumor antigen
Cancer patient
Tumor cell
Tumor antigen
Dead tumor cell
Complement-mediated tumor killing
Fc receptor–mediated phagocytosis and tumor killing
FcR
NK cell
NK-mediated ADCC
B Antibody specific for cell surface tumor antigen with conjugated drug
Internalization of drug into cell
Antibody delivery of chemotherapy drug to cancer cell
C Antibody specific for cell surface tumor signaling molecule
Tumor cell
Antibody block of growth factor receptor function
D Bispecific T cell engager (BiTE) specific for cell surface tumor antigen and CD3
TCR
Tumor antigen
CD3
CTL
Tumor cell
BiTE-mediated CTL killing of tumor

FIGURE 18.9 Mechanisms of action of antitumor antibodies.Many different monoclonal antibodies that bind directly to molecules on the surface of cancer cells are used as drugs to treat cancers. Some of the main mechanisms by which these antibodies work to kill cancer cells are shown. Other monoclonal antibodies used for cancer immunotherapy that target soluble growth factors or cell surface molecules on immune cells are not shown. *ADCC,* Antibody-dependent cellular cytotoxocity; *CTL,* cytotoxic T lymphocyte; *FcR,* Fc receptor; *NK,* natural killer cell; *TCR,* T cell receptor.

- Immunotoxins, or conjugated monoclonal antibodies, are antibodies specific for tumor antigens that are linked to a chemotherapy drug or a radioisotope (Fig. 18.9B). The rationale for these agents is they will allow high local concentrations of the cytotoxic drug or isotope to be delivered to the tumor cells, because of the antibody specificity. Several drug-conjugated antibodies are approved for clinical use, including those specific for HER2/NEU to treat breast cancers, CD30 to treat Hodgkin's lymphoma, CD33 to treat acute myeloid leukemia, CD22 to treat some acute lymphoblastic leukemias, and CD79b to treat some B cell lymphomas. Many more antibody-drug conjugates have been developed, but some have failed in clinical trials because of significant systemic toxicity due to the nonspecific accumulation of the toxic component in various tissues.

- Other monoclonal antibodies used in cancer therapy bind to growth factor receptors on cancer cells and interfere with the signaling required for tumor growth and survival (Fig. 18.9C). Anti-HER2/NEU is an approved monoclonal antibody used to treat breast cancers that overexpress the cell surface growth factor signaling molecule HER2/NEU. An antibody that binds and blocks the function of the epidermal growth factor receptor (EGFR) is approved for the treatment of metastatic colorectal cancers and head and neck cancers. Another antibody in clinical use for several cancers blocks an endothelial growth factor, VEGF, that stimulates the angiogenesis that is required to maintain tumor growth. In this case the antibody does not bind to the tumor cell.
- Bispecific T cell engagers (BiTEs) facilitate the targeting of host T cells of any specificity to attack tumor cells (Fig. 18.9D). These reagents are recombinant proteins engineered to express two different antigen binding sites, one specific for a tumor antigen and the second specific for a T cell surface molecule, usually CD3. In BiTEs, each antigen binding site is composed of a single chain variable fragment, which is a singe polypeptide containing tandem

immunoglobulin (Ig) heavy and light chain variable domains. The presumed mechanism of action of BiTEs, based on in vitro studies, is the formation of immune synapses between the tumor cells and the T cells and the activation of the T cells by CD3 ligation. A CD19-specific BiTE is approved for treatment of acute lymphocytic leukemia. BiTEs specific for many other tumor antigens have been developed, including CD20, BCMA, EpCAM, HER2/NEU, EGFR, CEA, folate receptor, and CD33, and are at various stages of preclinical development and clinical trials.

Adoptive Cellular Therapy With Antitumor T Cells

Adoptive cellular immunotherapy is the transfer of immune cells that have antitumor reactivity into a tumor-bearing host. The immune cells are derived from a cancer patient's blood or solid tumor and then are treated in various ways in vitro to expand their numbers and enhance their antitumor activity, before reinfusion back into the patient.

Adoptive Cellular Therapy With Autologous Tumor-Specific T Cells

T cells specific for tumor antigens can be harvested from a patient's tumor tissue or blood, expanded and activated in vitro, and infused back into the cancer patients (Fig. 18.10A) . This general approach has been used in various trials for many years, but has had limited success, probably because the cells that are isolated from patients contain a low frequency of potent tumor-specific T cells. With the advent of the technologies discussed earlier to identify the neoantigens that drive tumor-specific T cell responses in individual patients, there is renewed hope for adoptive therapy with T cells specific for these antigens. The approach will involve harvesting T cells from the blood or tumors of patients, stimulating the cells with tumor neoantigens in vitro to increase the numbers and functional activity of cells specific for the tumor, and then transferring the activated T cells back into the patient. There have already been some successes with small trials using this approach in patients with melanoma. Another T cell adoptive therapy approach currently in development is to transduce a patient's T cells to express T cell receptors (TCRs) specific for a tumor antigen commonly expressed by a particular tumor type and displayed by a known HLA (human leukocyte antigen) molecule. The genes encoding the TCR polypeptide chains are derived from cloned T cells taken from a patient with that tumor and are incorporated into a lentiviral expression vector that can be used to infect T cells taken from any patient with that tumor type and with the same HLA. This approach will require identifying antigens that are targets for T cells and expressed in the same type of tumor in different patients. The TCR genes may be optimized by introducing mutations that enhance the affinity of the TCRs for the antigen.

Chimeric Antigen Receptor T Cell Therapy

Adoptive therapy using T cells expressing chimeric antigen receptors (CARs) has proved successful in some hematologic malignancies, and this approach is being developed for other tumors (Fig 18.10B) **.** CARs are genetically engineered membrane-bound receptors with tumor antigen–specific binding sites encoded by recombinant Ig variable genes (i.e., single chain variable fragments) and cytoplasmic tails containing signaling domains of both the TCR complex and T cell costimulatory receptors (Fig. 18.11). The reason for using an antibody-like binding site as the tumor antigen recognition receptor is that this approach avoids the problem of the MHC restriction of TCRs, so the same CAR construct can be used for a particular tumor type in any patient. In addition, the CARs will be able to recognize tumor antigens even if the tumors stop expressing MHC molecules, which is a frequent mechanism of immune evasion. The genetically engineered cytoplasmic tails of CARs contain signaling domains that normally serve critical roles in T cell activation. Several variations of signaling constructs have been used so far in CARs developed at different centers, but all contain the TCR ζ chain immunoreceptor tyrosine-based activation motif (ITAM) motifs and the cytoplasmic signaling motifs of costimulatory receptors such as CD28 or CD137 (4-1BB). These signaling motifs confer on the tumor-specific Ig-like receptor the ability to potently activate T cells.

In current protocols, a cancer patient's peripheral blood T cells are isolated, infected with CAR-encoding retroviral or lentiviral vectors, and then stimulated with anti-CD3 and/or anti-CD28 antibodies to expand their numbers. The expanded CAR-expressing T cells are then injected back into the patient (see Fig. 18.10B). Prior to transfer, the patients are usually treated with drugs that deplete their own lymphocytes, which maximizes the proliferation of the transferred CAR-T cells. The transferred T cells undergo robust expansion in the patient, in response to tumor antigen recognition by the Ig domain of the CAR and costimulatory signals provided by the signaling domains. The specificities of the endogenous TCRs on these T cells (which are still present) become irrelevant to the goal of killing tumor cells, because all the CAR-expressing T cells can be activated by the tumor antigen that binds to the receptor encoded by the CAR gene. Tumor killing is achieved by both direct cytotoxic and cytokine-mediated mechanisms. CAR-T cells specific for CD19, a pan–B cell marker expressed on B cell–derived tumor cells, is approved for treatment of various B cell malignancies that are refractory to other treatments, including chronic lymphocytic leukemia, acute lymphoblastic leukemia, and B cell lymphomas, and CD20-specific CAR-T cells are approved for B cell lymphomas. Memory CAR-T cells may persist in the treated patients, so that surveillance against tumor recurrence is maintained. The technologies to produce large numbers of CAR-T cells for each patient in a short time have advanced greatly, and CAR-T cells are now used in many medical centers.

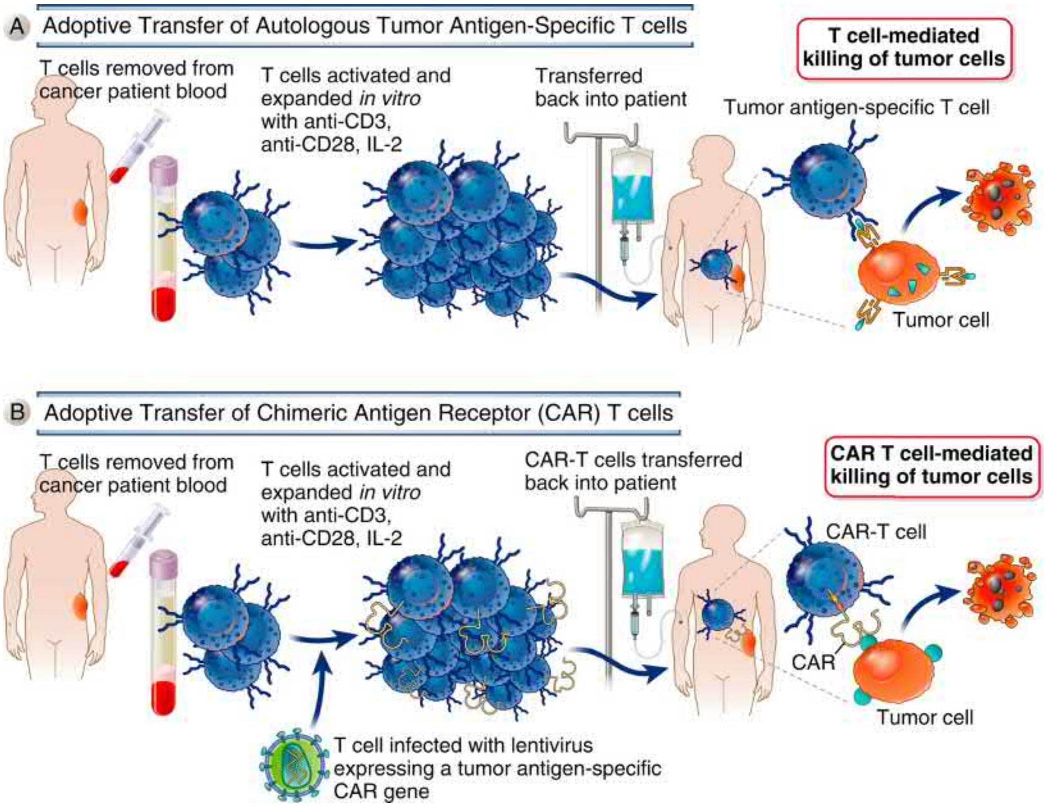

A
Adoptive Transfer of Autologous Tumor Antigen-Specific T cells
T cells removed from cancer patient blood
T cells activated and expanded *in vitro* with anti-CD3, anti-CD28, IL-2
Transferred back into patient
T cell-mediated killing of tumor cells
Tumor antigen-specific T cell
Tumor cell
B
Adoptive Transfer of Chimeric Antigen Receptor (CAR) T cells
T cells removed from cancer patient blood
T cells activated and expanded *in vitro* with anti-CD3, anti-CD28, IL-2
CAR-T cells transferred back into patient
CAR T cell-mediated killing of tumor cells
CAR-T cell
CAR
Tumor cell
T cell infected with lentivirus expressing a tumor antigen-specific CAR gene

FIGURE 18.10 Adoptive T cell therapy. **(A)** T cells isolated from the blood of a cancer patient are activated in vitro to expand their numbers and induce effector phenotypes and are then infused back into circulation of the same patient. Some of these T cells that are specific for tumor antigens and will be activated by and kill the tumor cells expressing those antigens. **(B)** Chimeric antigen receptors *(CARs)* are genetically engineered membrane-bound receptors that recognize tumor antigen by antibody-like binding sites and generate intracellular signals that activate T cells. CAR-T cells are generated by transducing a cancer patient's blood T cells in vitro with viruses engineered to express the CARs and are expanded in vitro before infusion back into the patient. CAR-T cell therapy has been successful

for treatment of certain leukemias and lymphomas. *IL-2,* interleukin-2.

There remain some significant roadblocks that will need to be overcome for successful expansion of the use of CAR-T cell therapy.

- The tumor antigens targeted by CAR-T cells may be expressed by some normal cells, which also will be killed. In the case of the first CARs in clinical use specific for CD19, normal B cells are killed, but, if necessary, patients can be supplemented with pooled Ig to make up for the lack of B cells. Because long-lived antibody-producing plasma cells found in adult bone marrow and mucosal tissues do not express CD19 and are not killed, they continue to provide antibody-mediated immunity in adult patients treated with CD19-specific CAR-T cells.
- Another problem commonly encountered in CAR-T cell therapy is cytokine release syndrome, a dangerous adverse reaction that frequently occurs soon after adoptive transfer of the T cells into patients with a high tumor burden. In these patients, so many of the T cells become activated at the same time that an intense systemic inflammatory response occurs due to the cytokines secreted by the T cells, which in turn stimulate cytokine release from macrophages and other cell types. Some patients who develop this reaction have been successfully treated with anti–IL-6 receptor antibody. The severity of this complication can be decreased by treating patients with cytotoxic chemotherapy to reduce tumor burden before CAR-T cell transfer. This limits the magnitude of the CAR-T cell activation that occurs after transfer. Other patients have developed serious neurotoxicity after CAR-T cell infusion, which may be related to microvascular injury or the effects of secreted cytokines that get into the brain, and the risk for long-term damage to the central nervous system remains a concern,

especially in children whose brains are incompletely developed.

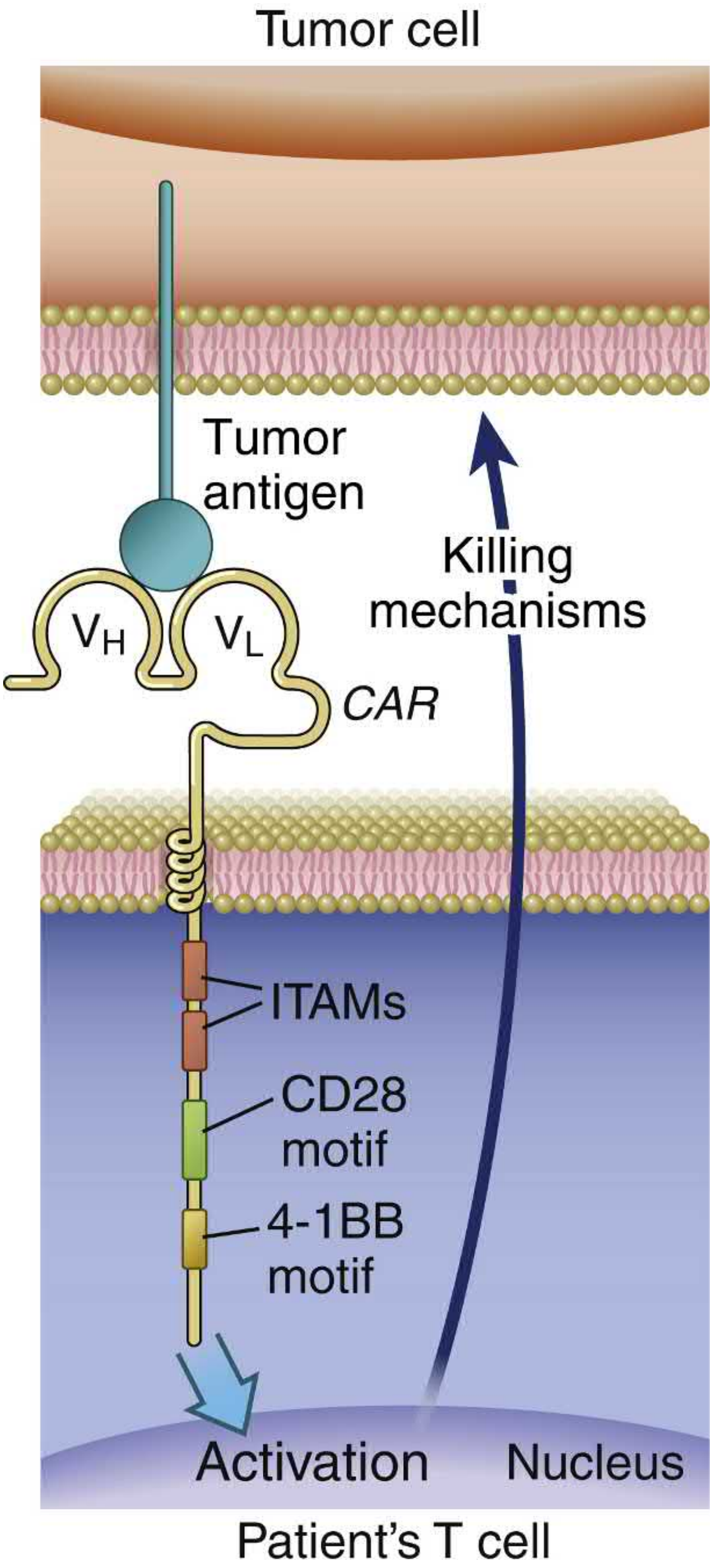

Tumor cell
Tumor antigen
Killing mechanisms
V_H
V_L
CAR
ITAMs
CD28 motif
4-1BB motif
Activation
Nucleus
Patient's T cell

FIGURE 18.11 Chimeric antigen receptor.Chimeric antigen receptors *(CARs)* are composed of an extracellular immunoglobulin single chain variable fragment specific for a tumor antigen, and cytoplasmic signaling domains that activate T cells, such as the ζ chain immunoreceptor tyrosine-based activation motif *(ITAMs)* and motifs in the cytoplasmic tails of the costimulatory receptors CD28 and 4-1BB, which promote robust T cell activation. V_H, V region of one heavy chain; V_L, V region of one light chain.

- Tumors may lose expression of the antigen being targeted by the CAR and recur. The selection pressure imposed by the CAR-T cells likely promotes the emergence of antigen-loss tumor variants. This problem may be reduced by simultaneously expressing CARs specific for two (or, theoretically, more) tumor antigens; the chance of a tumor clone losing multiple antigens is less.
- In some patients, transferred CAR-T cells appear to become unresponsive over time, and initially controlled tumors have recurred. The CAR-T cells in these patients express markers of exhaustion (see Chapter 11), including high levels of PD-1, and resemble the exhausted T cells mentioned earlier. This problem can be addressed by treating the CAR-T cell recipients with blocking antibodies specfic for PD-1 or by using genome editing methods to eliminate the PD-1 gene in the CAR-T cells. To avoid the risk for autoimmunity induced by the PD-1–negative T cells, endogenous TCRs are also eliminated from the T cells before transfer. This creates T cells that have only the introduced tumor-specific antigen receptor and do not become exhausted because they lack immune checkpoint molecules.

- Attempts to treat solid nonhematologic tumors with CAR-T cells have been hampered by difficulties in identifying optimal antigen targets for CAR-T cells on solid tumors that are not also expressed on normal cells whose destruction would cause serious toxicity. The reason for this may be that cell surface antigens, which have to be the targets of the CARs, are usually not tumor-specific and are often differentiation antigens or signaling receptors that are expressed at some level on normal cells of particular lineages. One approach to overcome this problem is to identify pairs of antigens that are commonly expressed together only on tumor cells and to transduce T cells with two different CARs, each specific for one of the antigens and each with different signaling domains, both of which have to be engaged to activate the T cells. It is hoped that antigens can be identified such that the likelihood of tumor cells expressing both antigens is greater than that of any normal cells expressing both. Getting the injected CAR-T cells to migrate into the sites of solid tumors is also a challenge. Strategies to genetically engineer the CAR-T cells with enhanced migratory functions are in development.

Studies are also ongoing to express CARs in cells other than $CD8^+$ T cells, such as NK cells, with the hope that these will be effective in killing tumors but will cause less toxicity than do CAR-T cells.

Immune Checkpoint Blockade: Targeting T Cell Inhibitory Pathways

Blockade of T cell inhibitory molecules has emerged as one of the most promising methods for effectively enhancing patients' immune responses to their tumors. This approach is based on the idea that tumor cells exploit various normal pathways of immune regulation or tolerance to evade the host immune response, as discussed earlier. Because these inhibitory mechanisms establish checkpoints in immune responses, the approach of stimulating immune responses by a drug that inhibits the inhibitors is called **immune checkpoint blockade** (Fig. 18.12). The first drug developed in this class is a monoclonal antibody specific for CTLA-4, the receptor on T cells that binds B7-1 and B7-2 and blocks T cell costimulation, especially during T cell priming in secondary lymphoid organs (see Chapter 15). Anti–CTLA-4 was first approved as a therapy for advanced melanoma, and it is effective in stopping or slowing tumor progression in many, but not a majority, of treated patients. This antibody may work by blocking the action of CTLA-4 expressed on activated T cells and on Tregs. As discussed earlier, T cell responses against tumors also may be inhibited by the PD-L1/PD-1 pathway, which works by activating a phosphatase that blocks the activation of effector T cells. Antibody blockade of PD-1 or its ligand PD-L1 appears to be even more effective than anti–CTLA-4 in enhancing T cell killing of tumors and halting the progression of otherwise lethal advanced cancers. Anti–PD-1 and anti–PD-L1 antibodies also cause less severe adverse effects (described later) than does anti–CTLA-4, and these antibodies are now approved for the treatment of many types of cancers, including melanoma, lung carcinomas, renal carcinomas, bladder carcinomas, colon carcinomas, Hodgkin's lymphoma, and others. In fact, anti–PD-1 is approved for all recurrent or metastatic tumors with mismatch repair defects, which result in high levels of mutations and thus production of abundant neoantigens in every histologic type of tumor. This is the first cancer therapy that has been approved based on a genetic signature of the tumor regardless of the tissue or cell of origin of the tumor. It is likely that the antitumor T cells that respond to this type of therapy in each patient are $CD8^+$ T cells that recognize neoantigen-derived peptides presented by class I MHC. Combined blockade of both PD-1 and CTLA-4 appears to be more effective against certain cancers than either alone and is approved for several cancers.

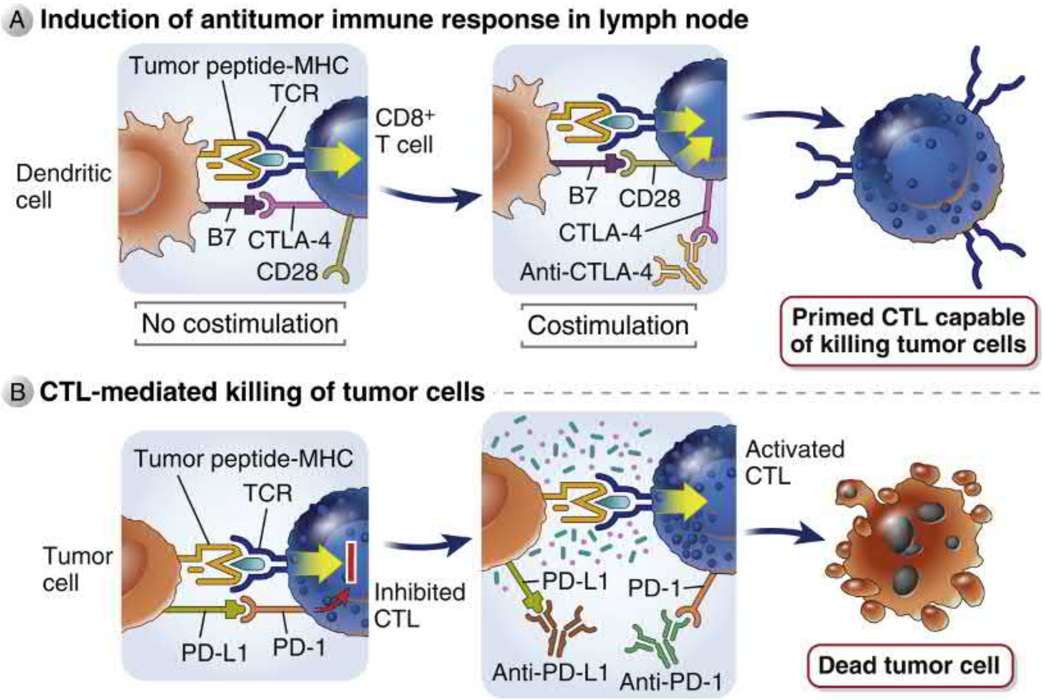

A Induction of antitumor immune response in lymph node
Tumor peptide-MHC
TCR
Dendritic cell
CD8+ T cell
B7
CTLA-4
CD28
No costimulation
B7
CD28
CTLA-4
Anti-CTLA-4
Costimulation
Primed CTL capable of killing tumor cells
B CTL-mediated killing of tumor cells
Tumor peptide-MHC
TCR
Tumor cell
PD-L1
PD-1
Inhibited CTL
PD-L1
PD-1
Anti-PD-L1
Anti-PD-1
Activated CTL
Dead tumor cell

FIGURE 18.12 Checkpoint blockade. Tumor patients often mount ineffective T cell responses to their tumors because of the upregulation of inhibitory receptors such as CTLA-4 and PD-1 on the tumor-specific T cells and expression of the ligand PD-L1 on the tumor cells. Blocking anti–CTLA-4 antibodies **(A)** or anti–PD-1 or anti–PD-L1 antibodies **(B)** are highly effective in treating several types of advanced tumors by releasing the inhibition of tumor-specific T cells by these molecules. Anti–CTLA-4 may work by blocking CTLA-4 on responding T cells *(shown)* or on regulatory T (Treg) cells. *CTLA-4,* Cytotoxic T lymphocyte antigen 4; *MHC,* major histocompatibility complex; *PD-1,* programmed cell death protein-1; *PD-L1,* PD ligand-1; *TCR,* T cell receptor.

Common adverse effects of checkpoint blockade treatment of cancers are autoimmune and inflammatory reactions, collectively called immune-related adverse events, which is predictable in light of the known roles of CTLA-4 and PD-1 in maintaining self-tolerance and regulating T cell responses. The most frequent adverse events involve inflammation of the colon, lung, liver, and various endocrine organs, although many other organs and tissues, including muscles, the heart, and central nervous system, can be affected. Some of these inflammatory disorders are unusual in the absence of checkpoint blockade, such as autoimmune destruction of the anterior pituitary gland, or acute-onset and rapid destruction of pancreatic islets, leading to insulin-dependent diabetes. Most of these adverse events can be successfully treated with antiinflammatory medications such as corticosteroids or corrected with hormone replacement therapy, but many require cessation of the checkpoint blockade therapy and some, such as myocarditis, have a high mortality rate.

Overall, only about 15% to 20% of patients with cancer treated with anti–CTLA-4 and/or anti–PD-1 have objective improvement in their disease, and, among these, some tumors recur after an initial response. There are several possible reasons for these therapeutic failures.

- Checkpoint blockade therapy is unlikely to work in patients with tumors that have relatively few somatic mutations encoding neoantigens because there will be few clones of tumor-specific T cells that will respond.
- Many tumors do not take advantage of the CTLA-4 or PD-1–PD-L1 pathway as a strategy to evade antitumor immunity, but rather employ other immune evasion mechanisms. Consistent with this concept, low levels of expression of PD-L1 on some tumor types, detected by immunohistochemistry, predict a poor response to anti–PD-1 therapy.
- PD-L1–expressing tumors that initially respond to anti–PD-1 therapy may become resistant in the presence of the strong immune response. The acquired resistance could occur by selective growth of clones of tumor cells that express molecules other than PD-L1 that inhibit T cell responses.

> Alternatively, clones of tumor cells may be selected that induce the T cells to express other checkpoint receptors besides PD-1.

An important goal of cancer immunologists and oncologists is to identify histologic or genetic features of tumors or circulating biomarkers that may predict which patients will respond best to which checkpoint blockade therapy. The nature of the cellular infiltrate around the tumor has some predictive value of the response to checkpoint blockade. In general, abundant effector T cells, even if they have the phenotype of dysfunctional (or exhausted) cells, predict a good response, whereas sparse cellular infiltrates or an abundance of Tregs predict poor responses. In the future, assays for T cells expressing antigen receptors (TCRs) specific for neoantigens may be combined with analysis of neoantigen abundance to provide greater predictive value. The presence of B cells in the tumor infiltrate also correlates with good responses to checkpoint blockade. It is not known if this is because B cells and antibodies contribute to antitumor immunity or if the presence of B cells implies a robust immune response against the tumor.

To increase the percentage of patients who respond to checkpoint blockade, oncologists are testing the efficacy of blocking other T cell inhibitory molecules, including LAG3, TIGIT, and TIM3, alone or in combination with anti–PD-1 or anti–CTLA-4. Other approaches include combining checkpoint blockade with more conventional radiation or chemotherapy protocols or combining checkpoint blockade with tumor vaccines (discussed later), kinase inhibitors that block oncogenic pathways in the tumors, or stimulating (agonistic) antibodies specific for activating receptors on T cells.

Vaccination With Tumor Antigens

Vaccination of tumor-bearing individuals with tumor antigens may result in enhanced immune responses against the tumor. The earliest attempts to boost antitumor immunity relied on nonspecific immune stimulation. More recently, vaccines composed of killed tumor cells, recombinant tumor antigens, or DCs incubated with tumor antigens have been tested in animal models and in clinical trials with patients with cancer.

The identification of peptides recognized by tumor-specific CTLs and the identification of mutant genes that encode tumor-specific antigens recognized by CTLs have provided many candidate antigens to include in tumor vaccines. New DNA sequencing technologies are now widely used to rapidly determine all the mutations in the protein-coding DNA sequences (exomes) of cancer cell genomes. Prediction algorithms are applied to these data to identify mutant peptides that are most likely to bind to the MHC alleles of each patient. These technical advances now allow for the precise identification of tumor-specific neoantigens in individual tumors, and this has stimulated efforts for the development of personalized tumor vaccines (Fig. 18.13).

Tumor vaccination strategies employ a variety of adjuvants and delivery methods.

- Proinflammatory molecules are used to enhance the numbers of activated DCs at the vaccination site. These adjuvants include Toll-like receptor (TLR) ligands, such as CpG DNA and mimics of double-stranded RNA (dsRNA), and cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-12.
- Tumor antigens are delivered in the form of DC vaccines (Fig. 18.14). In this approach, DCs are purified from patients, incubated with tumor antigens, and then injected back into the patients. A DC-based vaccine is now approved to treat advanced prostate cancer, but it has not proved to be effective in most patients. Technical challenges with DC vaccines are that the cells have to be harvested from each patient and they require expansion in cell culture, which is difficult to standardize.
- DNA vaccines and viral vectors encoding tumor antigens are being tested in clinical trials. These may be the best

ways to induce CTL responses because the encoded antigens are synthesized in the cytosol of cells, such as DCs, and efficiently enter the class I MHC pathway of antigen presentation.

Overall, the results of trials with many different types of tumor vaccines have been inconsistent and generally not very successful. Unlike many standard vaccines against microbes, which prophylactically prevent infections, tumor vaccines are used as therapies that need to be effective in stopping progression of tumors that have already developed. Most tumor vaccines tried to date have targeted antigens that are commonly shared by the same tumor type in different patients, and these antigens are usually differentiation antigens also expressed on cells of the normal tissue from which the cancer arose. Vaccines using such antigens have generally not been successful, likely because the antigens in normal cells induce tolerance that has to be overcome for induction of effective antitumor immunity.

The development of virus-induced tumors can be reduced by preventive vaccination with viral antigens or attenuated live viruses. As mentioned earlier, HPV vaccines have been effective in decreasing the incidence of HPV-induced premalignant lesions and cancers in the cervix. This approach has also been extremely successful in reducing the incidence of feline leukemia virus–induced hematologic cancers in cats and in preventing Marek's disease, a herpes virus–induced lymphoma, in chickens.

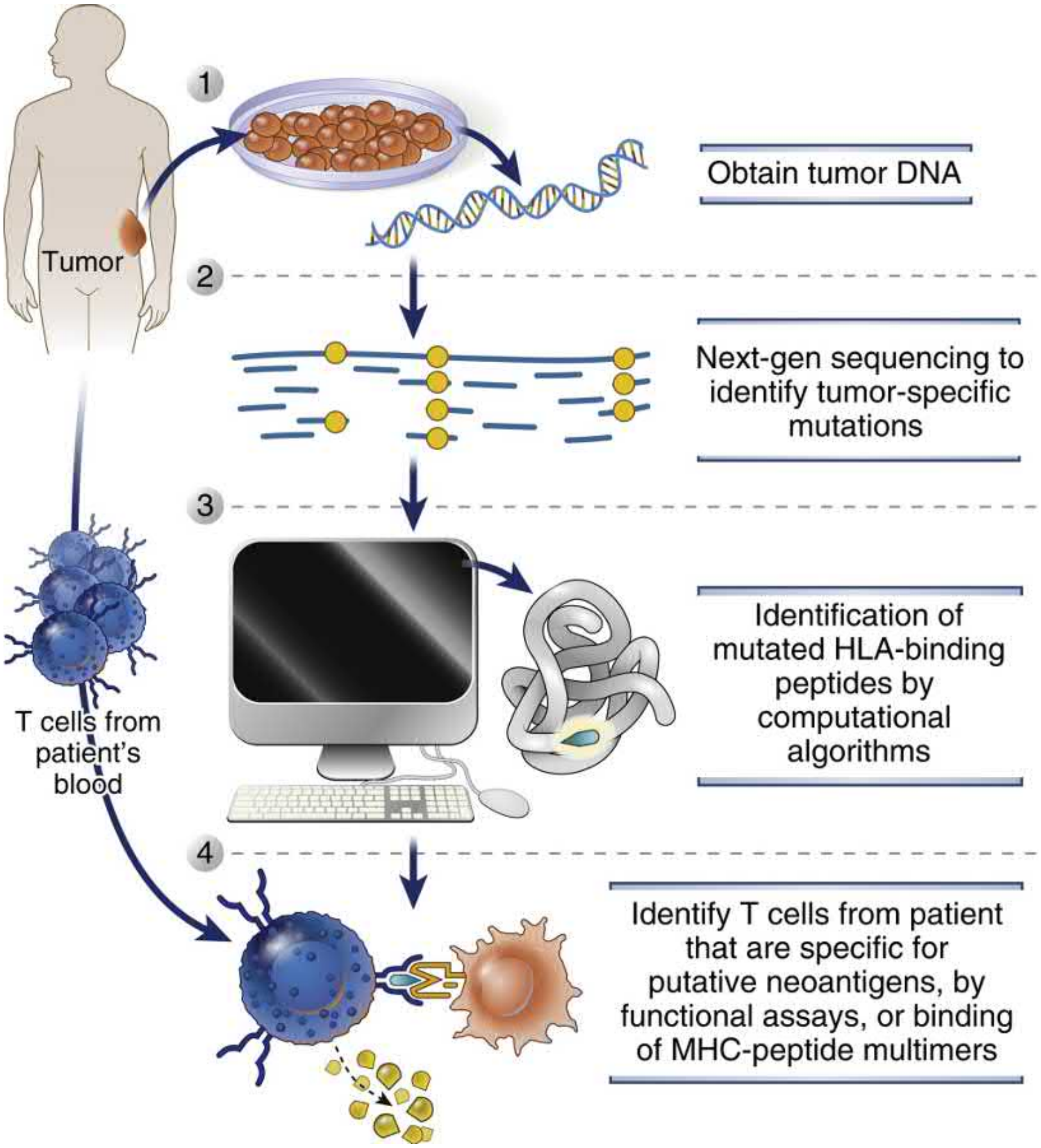

1
2
3
4
Tumor
T cells from patient's blood
Obtain tumor DNA
Next-gen sequencing to identify tumor-specific mutations
Identification of mutated HLA-binding peptides by computational algorithms
Identify T cells from patient that are specific for putative neoantigens, by functional assays, or binding of MHC-peptide multimers

FIGURE 18.13 Detecting tumor neoantigens that elicit T cell responses. Tumor DNA can be purified *(1),* and exome sequencing can detect random mutations in the genome of cancer cells *(2).* A computer algorithm then can be used to determine which mutations occur in amino acid sequences that encode peptides that would bind to the major histocompatibility complex *(MHC)* alleles in that patient *(3).* The validity of the putative neoantigenic peptides can be tested by assays of patient T cell response to these peptides in vitro

or by testing if MHC-peptide multimeric complexes can bind to the T cells *(4).* This approach is being used to create personalized tumor vaccines. *gen,* Generation; *HLA,* human leukocyte antigen.

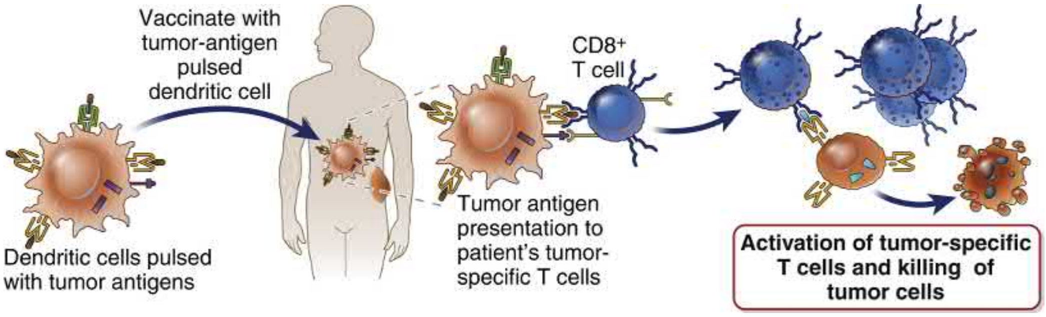

Vaccinate with tumor-antigen pulsed dendritic cell
CD8+ T cell
Dendritic cells pulsed with tumor antigens
Tumor antigen presentation to patient's tumor-specific T cells
Activation of tumor-specific T cells and killing of tumor cells

FIGURE 18.14 Dendritic cell vaccines.Dendritic cells (DCs), generated in vitro from blood monocytes taken from a patient with a tumor can be pulsed with defined tumor antigens and infused back into the patient, where they will present the antigen to T cells specific for that antigen and boost a tumor-specific immune response. In other approaches, the DCs are transfected with a gene encoding the tumor antigen and sometimes also a cytokine that promotes immune responses, and these cells are used as vaccines.

Other Approaches for Stimulating Antitumor Immunity

Several additional approaches have been used to enhance host immunity against tumors, with variable success.

Cytokine Therapy

Cancer patients can be treated with cytokines that stimulate the proliferation and differentiation of T lymphocytes and NK cells.

These cytokines can enhance the activation of DCs and tumor-specific T cells, particularly CD8$^+$ CTLs. Many cytokines also have the potential to induce nonspecific inflammatory responses, which by themselves may have antitumor activity. The largest clinical experience is with high-dose IL-2 given intravenously, which has been effective in inducing measurable tumor regression in about 10% of patients with advanced melanoma and renal cell carcinoma and is currently an approved therapy for these cancers. The use of high-dose IL-2 is, however, limited because it stimulates the production of toxic amounts of proinflammatory cytokines such as TNF and IFN-γ, which act on vascular endothelial and other cells and lead to a serious vascular leak syndrome.

IFN-α is approved for treatment of several cancers, including malignant melanoma, certain lymphomas and leukemias, and AIDS-related Kaposi sarcoma. The mechanisms of the antineoplastic effects of IFN-α probably include inhibition of tumor cell proliferation, increased cytotoxic activity of NK cells, and increased class I MHC expression on tumor cells, which makes them more susceptible to killing by CTLs.

Other cytokines, such as TNF and IFN-γ, are effective antitumor agents in animal models, but their use in patients is limited by their toxic side effects. Hematopoietic growth factors, including GM-CSF and G-CSF, are used in cancer treatment protocols to shorten periods of neutropenia and thrombocytopenia after chemotherapy or autologous bone marrow transplantation.

Oncolytic Viruses

Oncolytic viruses are genetically modified viruses that selectively replicate in and cause lytic death of cancer cells, and, in doing so, promote CTL responses specific for tumor antigens that are released. The first oncolytic virus approved for clinical use is talimogene laherparepvec (TVEC) to treat metastatic melanoma. TVEC is a herpes simplex virus in which genes have been deleted to enhance viral-induced cell lysis and reduce chronic infection of nerves. Furthermore, a gene that encodes a TAP inhibitor has been deleted, thus enhancing the class I MHC antigen presentation pathway in infected cells, and a gene encoding GM-CSF has been added, which increases DC accumulation in the tumor microenvironment. TVEC is injected directly into solid tumor sites but enhances antitumor CTL responses even at distant locations from the injection site, which is evidence that the oncolytic virus promotes systemic antitumor immunity. Clinical trials are being conducted to test the efficacy of TVEC on other tumor types, and other oncolytic viruses are in development.

Nonspecific Inflammatory Stimuli

Immune responses to tumors may be stimulated by the local administration of inflammatory substances or by systemic treatment with agents that function as polyclonal activators of lymphocytes. One of the oldest examples of tumor immunotherapy was practiced by the 19th-century physician William Coley, who treated his cancer patients with extracts of dead bacteria, so called Coley's toxin. This approach may have been intermittently successful due to the induction of strong innate responses leading to production of TNF and other cytokines that caused acute inflammation that killed tumor cells. Nonspecific immune stimulation of patients with tumors by injection of inflammatory substances such as killed bacillus Calmette-Guérin (BCG) at the sites of tumor growth has been used for many years. The BCG mycobacteria activate macrophages and thereby promote macrophage-mediated killing of the tumor cells. In addition, the bacteria function as adjuvants and may stimulate T cell responses to tumor antigens. Intravesicular BCG is currently used to treat bladder cancer. Cytokine therapies, discussed earlier, represent another method of enhancing immune responses in a nonspecific manner.

Graft-Versus-Leukemia Effect

In leukemia patients treated by allogeneic hematopoietic stem cell (HSC) transplant, the presence of T cells and NK cells in the HSC inoculum can contribute to eradication of the tumor. The T cell–

mediated graft-versus-leukemia effect is directed at molecules present on the recipient's hematopoietic cells, including the leukemia cells, that are recognized as foreign by the administered T cells. Donor NK cells respond to the tumor cells because tumors may express low levels of class I MHC molecules or they express class I MHC alleles not recognized by the donor NK cells. Recall that recognition of self class I MHC normally inhibits the activation of NK cells (see Chapter 4). The challenge in use of this treatment to improve clinical outcome is to minimize the dangerous graft-versus-host disease that may be mediated by the same donor T cells (see Chapter 17).

The remarkable recent advances in cancer immunotherapy promise to dramatically change the care of patients with these dreaded diseases. The success of checkpoint blockade for many solid tumors and of CAR-T cell infusion for hematologic malignancies has revitalized the field of tumor immunology. Although limitations and problems remain, the enormous effort being invested in this field makes it likely that further advances will happen rapidly.

Summary

- Tumors express antigens that are recognized by the immune system, but most tumors suppress immune responses or are weakly immunogenic, and immune responses often fail to prevent the growth of tumors. Nonetheless, the immune system can be therapeutically stimulated to effectively kill tumors.
- Tumor antigens recognized by cytotoxic T lymphocytes (CTLs) are the principal inducers of and targets for antitumor immunity. Tumor-specific neoantigens generated by random mutations of cellular proteins, which can be processed into major histocompatibility complex (MHC) binding mutant peptides, are the most important, but other tumor antigens known to stimulate host T cells include

products of mutated oncogenes, normal proteins whose expression is dysregulated or increased in tumors, and antigens of oncogenic viruses.

- Antibodies specific for tumor cell antigens are used for diagnosis, and the antigens are potential targets for antibody therapy. These antigens include oncofetal antigens, which are expressed normally during fetal life and whose expression is dysregulated in some tumors, altered surface glycoproteins and glycolipids, and molecules that are normally expressed on the cells from which the tumors arise and are thus differentiation antigens for particular cell types.
- Immune responses that are capable of killing tumor cells are mediated by CTLs, natural killer (NK) cells, and macrophages, possibly activated by tumor antigen–specific helper T cells. Among these immune effector mechanisms, the role of CTLs in protecting individuals from tumors is best defined.
- Tumors evade immune responses by several mechanisms, including downregulated expression of MHC molecules, selective outgrowth of cells that do not express tumor antigens, production of soluble immunosuppressive substances, the engagement of inhibitory receptors on lymphocytes by their ligands expressed on the tumor cells, and the induction of regulatory T cells. Tumor-associated macrophages and myeloid-derived suppressor cells, found in most solid tumors, can suppress antitumor immunity.
- Immunotherapy for tumors includes approaches that augment active immune responses against these tumors or provision of tumor-specific immune effectors to provide passive immunity to the patients. Antitumor immunity may be enhanced by blocking mechanisms of immune regulation. Immune responses also may be actively stimulated by vaccination with tumor cells or antigens, and by systemic administration of cytokines that stimulate immune responses.

- Antitumor antibodies are used widely in tumor immunotherapy. The antibodies bind to molecules on the surface of tumor cells and engage effector mechanisms to kill the tumors, including complement, NK cells, and phagocytes, or the antibodies bind to growth factor receptors, which blocks the signaling needed to sustain tumor cell growth. Tumor antigen–specific antibodies also have been conjugated with chemotherapeutic toxins or radioisotopes, to target these agents specially to tumors. Some genetically engineered bispecific antibodies simultaneously bind tumor antigens and activation receptors on T cells.
- CAR-T cell therapy is a form of cancer therapy in which a patient’s T cells are engineered ex vivo to express a hybrid antigen receptor (chimeric antigen receptor [CAR]) that recognizes a tumor antigen by antibody V domains and signals via cytoplasmic T cell receptor and costimulatory receptor motifs. The CAR-T cells are then transferred back to the tumor patient, where they become activated by tumor antigens and kill the tumor cells. CAR-T cell therapy has been effective in treating some hemopoietic tumors.
- Immune checkpoint blockade is a mode of tumor immunotherapy in which function-blocking antibodies against inhibitory receptors on T cells or their ligands, including PD-1 (programmed cell death protein-1), PD-L1 (PD-ligand 1), and CTLA-4 (cytotoxic T lymphocyte antigen 4), are administered to remove the brakes on lymphocyte activation and thus promote antitumor immunity by previously inhibited host T cells specific for tumor antigens. Checkpoint blockade has been widely adopted to treat many types of cancers.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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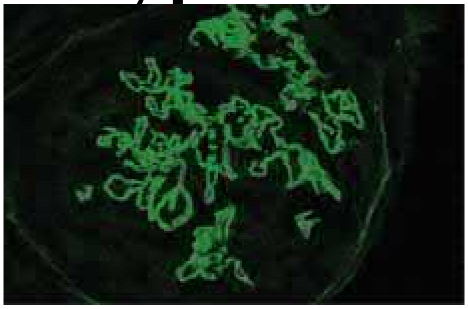
Chapter 19: Hypersensitivity Disorders



Causes of Hypersensitivity Diseases,

Mechanisms and Classification of Hypersensitivity Reactions,

Diseases Caused by Antibodies and Antigen-Antibody Complexes,
  Diseases Caused by Antibodies Against Fixed Cell and Tissue Antigens,
  Immune Complex–Mediated Diseases,

Diseases Caused by T Lymphocytes,
  Diseases Caused by Cytokine-Mediated Inflammation,
  Diseases Caused by Cytotoxic T Lymphocytes,

Therapeutic Approaches for Immunologic Diseases,

Selected Immunologic Diseases: Pathogenesis and Therapeutic Strategies,

  - Systemic Lupus Erythematosus (SLE): The Prototypic Immune Complex–Mediated Disease,
  - Rheumatoid Arthritis (RA),
  - Multiple Sclerosis (MS),
  - Type 1 Diabetes,
  - Inflammatory Bowel Disease,
  - Celiac Disease,
  - Psoriasis,

- Summary,

The immune system serves the important function of host defense against microbial infections, but immune responses are also capable of causing tissue injury and disease. Disorders caused by immune responses are called **hypersensitivity diseases.** This term arose from the clinical definition of immunity as sensitivity, which is based on the observation that an individual who has been exposed to an antigen exhibits a detectable reaction, or is sensitive, to subsequent encounters with that antigen. Pathologic, or injurious and excessive, reactions were then called hypersensitivity. Normally, immune responses eradicate infectious pathogens without serious injury to host tissues. However, these responses are sometimes inadequately controlled, inappropriately targeted to host tissues, or triggered by commensal microorganisms or environmental antigens that are usually harmless. In these situations, the normally beneficial immune response is the cause of disease.

In this chapter, we will describe the pathogenesis of different types of hypersensitivity reactions, with an emphasis on the effector mechanisms that cause tissue injury. We will conclude with a brief consideration of the treatment of immunologic diseases and examples of diseases that illustrate important principles.

Causes of Hypersensitivity Diseases

Hypersensitivity reactions may be specific for different types of antigens.

- ***Reactions against self antigens: autoimmunity.*** Failure of the normal mechanisms of self-tolerance results in T cell and B cell reactions against one's own cells and tissues that are called **autoimmunity** (see Chapter 15), and the diseases caused by these reactions are referred to as **autoimmune diseases.** Autoimmune diseases are estimated to affect at least 5% of the population in higher-income countries, and the incidence of these disorders is rising. Many of these diseases are more common in women than in men; the mechanisms underlying this gender bias remain obscure. Autoimmune diseases are usually chronic and often debilitating and are an enormous medical and economic burden. Although these disorders have been difficult to treat in the past, many new effective therapies have been developed since the 1990s based on scientific advances in immunology. The mechanisms of autoimmunity were described in Chapter 15. In this chapter, we will refer to various autoimmune disorders to illustrate how immune reactions against self cause disease.
- ***Reactions against microbes.*** Immune responses against microbial antigens may cause disease if the reactions are excessive or the microbes are unusually resistant to eradication and thus the infections are persistent. Ongoing T cell responses against persistent microbes may give rise to severe inflammation, sometimes with the formation of granulomas; this is the cause of tissue injury in tuberculosis and some other chronic infections. If antibodies are produced against microbial antigens, the antibodies may bind to the antigens to produce immune complexes, which deposit in tissues and trigger inflammation. Rarely, antibodies or T cells against a microbe will cross-react with

self antigens and cause damage to host tissues. In some cases involving the intestinal tract (i.e., inflammatory bowel disease [IBD]), the immune response may be directed against commensal bacteria that normally reside in the gut and cause no harm. Sometimes the mechanisms that an immune response uses to eradicate a pathogenic microbe require killing infected cells, and therefore such responses inevitably injure host tissues. For example, in hepatitis B virus infection, the virus that infects liver cells is not cytopathic, but it is recognized as foreign by the immune system. Cytotoxic T lymphocytes (CTLs) eliminate infected cells, and this normal immune response damages the liver. This type of normal reaction is not considered hypersensitivity.

- ***Reactions against nonmicrobial environmental antigens.*** Most healthy individuals do not react against common, generally harmless environmental substances, but 20% or more of the population is abnormally responsive to one or more of these substances. These individuals produce IgE (immunoglobulin E) antibodies that cause allergic diseases (see Chapter 20). Some individuals become sensitized to environmental antigens and chemicals that contact the skin and develop T cell reactions that lead to cytokine-mediated inflammation, resulting in contact sensitivity. Idiosyncratic immunologic reactions against therapeutic drugs are also a frequent clinical problem.

In all of these conditions, the mechanisms of tissue injury are the same as those that normally function to eliminate infectious pathogens. These mechanisms include innate and adaptive immune responses involving phagocytes, antibodies, T lymphocytes, mast cells, and other leukocytes and mediators of inflammation. The problem in hypersensitivity diseases is that the immune response is not controlled appropriately or is targeted to normal tissues. Because the stimuli for these abnormal immune responses are often impossible to eliminate (e.g., self antigens, commensal microbes, and environmental antigens) and the immune system has many built-in positive feedback loops (amplification mechanisms), once a pathologic immune response starts, it is difficult to control or to terminate it. Therefore, these hypersensitivity diseases tend to be chronic and progressive and pose major therapeutic challenges in clinical medicine.

In clinical situations, the term hypersensitivity is generally used to describe to harmful immune responses against foreign antigens (e.g., environmental antigens, drugs, microbes). However, in our discussion, we will consider all causes of harmful immune reactions, mainly to emphasize the common pathogenic mechanisms.

Mechanisms and Classification of Hypersensitivity Reactions

Hypersensitivity diseases are commonly classified according to the type of immune response and the effector mechanism responsible for cell and tissue injury (Table 19.1)**.** These mechanisms include some that are predominantly dependent on antibodies and others predominantly dependent on T cells, although humoral and cell-mediated immunities often coexist and both contribute to tissue injury in many hypersensitivity diseases.

- **Immediate (type I) hypersensitivity** is caused by IgE antibodies specific for nonmicobial environmental antigens and is the most prevalent type of hypersensitivity disease; it will be discussed in detail separately in Chapter 20. Immediate hypersensitivity diseases, commonly grouped under **allergy** or **atopy,** are often caused by activation of interleukin-4 (IL-4), IL-5, and IL-13 producing Th2 cells and the production of IgE antibodies, which activate mast cells and eosinophils and induce inflammation.
- **Antibody-mediated (type II) hypersensitivity.** IgG and IgM antibodies specific for cell surface or extracellular matrix

antigens can cause tissue injury by activating the complement system, targeting cells for phagocytosis by leukocytes, recruiting inflammatory cells, and interfering with normal cellular functions.

- **Immune complex–mediated (type III) hypersensitivity.** IgM and IgG antibodies specific for soluble antigens in the blood form complexes with the antigens, and the immune complexes may deposit in blood vessel walls in various tissues, causing inflammation, thrombosis, and tissue injury.
- **T cell–mediated (type IV) hypersensitivity.** In these disorders, tissue injury may be due to $CD4^+$ T lymphocytes, which secrete cytokines that induce inflammation, or $CD8^+$ CTLs, which kill target cells.

This classification is useful because distinct types of pathologic immune responses show different patterns of tissue injury and may vary in their tissue specificity. As a result, the different immunologic mechanisms cause disorders with distinct clinical and pathologic features. However, immunologic diseases in humans are often complex and caused by combinations of humoral and cell-mediated immune responses and multiple effector mechanisms. This complexity is not surprising, given that a single antigen may stimulate both humoral and cell-mediated immune responses in which several types of antibodies and effector T cells are produced. In the discussion that follows, we will use descriptions that identify the pathogenic mechanisms rather than the less informative numerical designations for types of hypersensitivity.

Diseases Caused by Antibodies and Antigen-Antibody Complexes

Antibody-mediated diseases are caused either by antibodies that bind to antigens on particular cells or in extracellular tissues or by antigen-antibody complexes that form in the circulation and are

deposited in vessel walls. Antibodies against cellular or tissue antigens result in diseases in which the immunologic injury affects the cells or tissues where these antigens are present, so these diseases are often organ-specific and not systemic. By contrast, the manifestations of diseases caused by immune complexes reflect the site of immune complex deposition and are not determined by the cellular source of the antigen. Therefore, immune complex–mediated diseases tend to be systemic and affect multiple tissues and organs.

TABLE 19.1

Type of Hypersensitivity	Pathologic Immune Mechanisms	Mechanisms of Tissue Injury and Disease
Immediate: Type I	IgE antibody, Th2 cells	Mast cells, eosinophils, and their mediators (vasoactive amines, lipid mediators, proteolytic enzymes, cytokines)
Antibody-mediated: Type II	IgM, IgG antibodies against cell surface or extracellular matrix antigens	Opsonization and phagocytosis of cells Complement- and Fc receptor–mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, for example, hormone receptor signaling, neurotransmitter receptor blockade
Immune complex–mediated: Type III	Immune complexes of circulating antigens and IgM or IgG antibodies	Deposition in blood vessel walls and tissuesComplement-mediated and Fc receptor–mediated recruitment and activation of leukocytes
T cell–mediated: Type IV	1. CD4$^+$ T cells (Th1 and Th17 cells) 2. CD8$^+$ CTLs	1. Cytokine-mediated inflammation and macrophage activation 2. Direct target cell killing, cytokine-mediated inflammation

CTLs, Cytotoxic T lymphocytes; *Ig,* immunoglobulin.

To prove that a disease is caused by antibodies, one would need to demonstrate that the lesions can be induced in a normal animal by the adoptive transfer of Ig purified from the blood or affected tissues of individuals with the disease. An experiment of nature is occasionally seen in children of mothers with antibody-mediated diseases. These infants may be born with transient manifestations of such diseases because of transplacental passage of antibodies. However, in clinical situations, the diagnosis of diseases caused by antibodies or immune complexes is usually based on the demonstration of antibodies or immune complexes in the circulation or deposited in tissues, as well as clinicopathologic similarities with experimental diseases that are proved to be antibody mediated by adoptive transfer.

Diseases Caused by Antibodies Against Fixed Cell and Tissue Antigens

Antibody-mediated diseases are produced by antibodies that bind to antigens on particular cells or in extracellular tissues. Antibodies against cell and tissue antigens cause disease by three main mechanisms:

- ***Opsonization and phagocytosis*** (Fig. 19.1A) **.** Antibodies that bind to surface antigens on circulating cells may opsonize these cells, or they may activate the complement system, resulting in the production of complement proteins that opsonize the cells. These opsonized cells are phagocytosed and destroyed by phagocytes that express receptors for the Fc portions of IgG antibodies and receptors for complement proteins. This is the principal mechanism of cell destruction in autoimmune hemolytic anemia and autoimmune thrombocytopenia, in which antibodies specific for red blood cells or platelets, respectively, lead to the opsonization and removal of these cells from the circulation. Splenectomy is beneficial in these diseases because the spleen is a major organ where opsonized cells are cleared by phagocytosis and where antibodies are produced. Antibody-coated red cells and platelets may also be lysed by the membrane attack complex of complement. The same mechanisms are responsible for hemolysis in transfusion reactions (see Chapter 17).
- ***Inflammation.*** Antibodies deposited in tissues activate complement, leading to the liberation of breakdown products such as C5a and C3a, which recruit neutrophils and macrophages (Fig. 19.1B). These leukocytes express IgG Fc receptors and complement receptors, which bind the antibodies or attached complement proteins. The leukocytes are activated by signaling from the receptors (particularly Fc receptors), and leukocyte products

(including lysosomal enzymes and reactive oxygen species) are released and cause tissue injury. Free antibodies most often deposit in basement membranes and extracellular matrix. An example of antibody-mediated inflammation and leukocyte activation causing tissue injury is glomerulonephritis caused by antibodies against the glomerular basement membrane (called Goodpasture syndrome if the antibodies also bind to basement membranes in the lungs).

- ***Abnormal cellular functions.*** Antibodies that bind to normal cellular receptors or other proteins may interfere with the functions of these receptors or proteins and cause disease without inflammation or tissue damage (Fig. 19.1C). For instance, antibodies specific for the thyroid-stimulating hormone receptor or the nicotinic acetylcholine receptor cause functional abnormalities that lead to Graves' disease and myasthenia gravis, respectively. Antibodies specific for intrinsic factor, required for vitamin B_{12} absorption, cause pernicious anemia. Antibodies specific for cytokines are rare but known causes of immunodeficiencies.

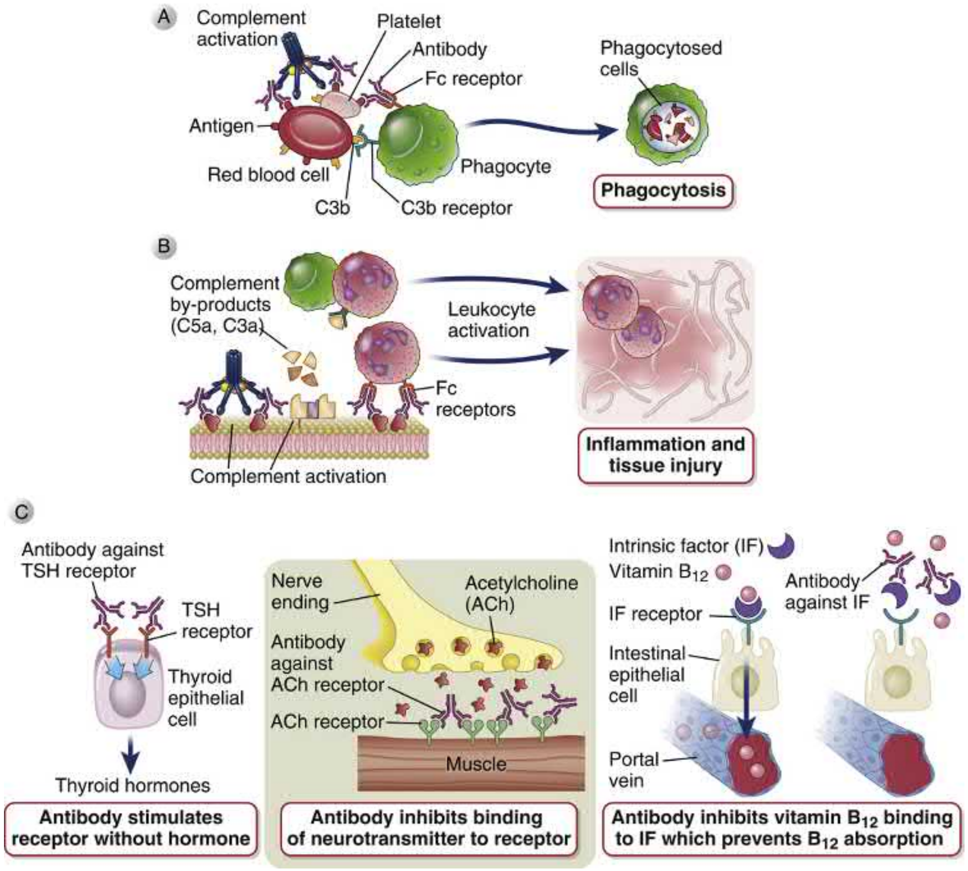

A
Complement activation
Platelet
Antibody
Fc receptor
Phagocytosed cells
Antigen
Red blood cell
C3b
C3b receptor
Phagocyte
Phagocytosis
B
Complement by-products (C5a, C3a)
Leukocyte activation
Fc receptors
Complement activation
Inflammation and tissue injury
C
Antibody against TSH receptor
TSH receptor
Thyroid epithelial cell
Thyroid hormones
Antibody stimulates receptor without hormone
Nerve ending
Acetylcholine (ACh)
Antibody against ACh receptor
ACh receptor
Muscle
Antibody inhibits binding of neurotransmitter to receptor
Intrinsic factor (IF)
Vitamin B_{12}
Antibody against IF
IF receptor
Intestinal epithelial cell
Portal vein
Antibody inhibits vitamin B_{12} binding to IF which prevents B_{12} absorption

FIGURE 19.1 Effector mechanisms of antibody-mediated disease. **A,** Opsonization and phagocytosis. Antibodies opsonize cells and may activate complement, generating complement products that also opsonize cells, leading to phagocytosis of the cells through phagocyte Fc receptors or C3b receptors. **B,** Inflammation. Antibodies recruit leukocytes by binding to Fc receptors or by activating complement and thereby releasing by-products that are chemotactic for leukocytes. **C,** Functional abnormalities. Antibodies specific for cell surface hormone receptors, neurotransmitter receptors, or secreted proteins interfere with normal physiology. For example, in Graves ‘disease *(left panel)* autoantibodies specific for thyroid

stimulating hormone (TSH) receptors in the thyroid gland stimulate the activity of the receptors even in the absence of TSH, causing excess thyroid hormone release (hyperthyroidism). In myasthenia gravis *(middle panel)*, autoantibodies specific for the acetylcholine receptor on muscle cells block the action of acetylcholine, leading to paralysis. In autoimmune pernicious anemia *(right panel),* antibodies against intrinsic factor interfere with the absorption of vitamin B_{12}, resulting in a deficiency that impairs hematopoiesis and causes anemia.

Antibodies that cause cell- or tissue-specific diseases are usually autoantibodies produced as part of an autoimmune reaction, but sometimes the antibodies are specific for microbes. Examples of autoantibodies against tissue antigens are listed in Table 19.2. Less commonly, the antibodies may be produced against a foreign (e.g., microbial) antigen that is immunologically cross-reactive with a component of self tissues. In a rare sequel to streptococcal infection called rheumatic fever, antibodies produced against the bacteria cross-react with antigens in the heart, deposit in this organ, and cause inflammation and tissue damage. Tissue deposits of antibodies may be detected by pathologic examination in some of these diseases, and the deposition of antibody is often associated with local complement activation, inflammation, and tissue injury (Fig. 19.2A).

TABLE 19.2

Disease	Target Antigen	Mechanisms of Disease	Clinicopathologic Manifestations
Autoimmune hemolytic anemia	Erythrocyte membrane proteins (various); chemicals (therapeutic drugs) attached to red cell proteins	Opsonization and phagocytosis of erythrocytes, complement-mediated lysis	Hemolysis, anemia
Autoimmune thrombocytopenic purpura	Platelet membrane proteins (e.g. gpIIb-IIIa integrin)	Opsonization and phagocytosis of platelets	Bleeding
Pemphigus vulgaris	Proteins in intercellular junctions of epidermal cells (desmoglein)	Antibody-mediated activation of proteases, disruption of intercellular adhesions	Skin blisters (bullae)
Vasculitis caused by ANCA	Neutrophil granule proteins, presumably released from activated neutrophils	Neutrophil degranulation and inflammation	Vasculitis
Goodpasture syndrome	Noncollagenous NC1 protein of basement membrane in glomeruli and lung	Complement- and Fc receptor–mediated inflammation	Nephritis, lung hemorrhage
Acute rheumatic fever	Streptococcal cell wall antigen; antibody cross-reacts with myocardial antigen	Inflammation, macrophage activation	Myocarditis
Myasthenia gravis	Nicotinic acetylcholine receptor	Antibody inhibits acetylcholine binding, downmodulates receptors	Muscle weakness, paralysis
Graves' disease (hyperthyroidism)	TSH receptor	Antibody-mediated stimulation of TSH receptors	Hyperthyroidism
Autoimmune pernicious anemia	Intrinsic factor secreted by gastric parietal cells	Neutralization of intrinsic factor; decreased absorption of vitamin B_{12}	Abnormal erythropoiesis, anemia, neurologic symptoms

ANCA, Anti-neutrophil cytoplasmic antibodies; *TSH,* thyroid-stimulating hormone.

Immune Complex–Mediated Diseases

Immune complex-mediated diseases are usually caused by antigen-antibody complexes that form in the circulation and are deposited in multiple tissues, producing systemic disorders (Fig. 19.3). The immune complexes that cause disease may be composed of antibodies bound to either self antigens or foreign antigens. Almost all of these diseases are systemic, but a few are restricted to kidneys, perhaps because, in those cases, complexes are formed only in the glomerular basement membrane.

The occurrence of diseases caused by immune complexes was suspected in the early 1900s by an astute physician named Clemens von Pirquet. At the time, diphtheria infections were treated with serum from horses that had been immunized with the diphtheria toxin, which is an example of passive immunization against the toxin by the transfer of serum containing antitoxin antibodies. Von Pirquet noted that joint inflammation (arthritis), rash, and fever developed in patients who were repeatedly injected with the antitoxin-containing horse serum. Clinical features of this reaction suggested that it was not due to the infection or a toxic component of the serum itself. The symptoms appeared at least 1 week after the first injection of horse serum and more rapidly with each repeated injection. Von Pirquet concluded that this disease was caused by a host response to some component of the serum. He suggested that the host made antibodies to horse serum proteins; these antibodies formed complexes with the injected proteins, and the disease was due to the antibodies or immune complexes. We now know that his conclusions were entirely accurate. He called this disorder serum disease; it is now known as serum sickness. It remains a clinical issue today in individuals who receive therapeutic antibodies produced in animals that contain nonhuman sequences, such as antisera used to treat snakebites or rabies or anti-thymocyte globulin used to suppress graft rejection.

Experimental Models of Immune Complex–Mediated Diseases

Serum Sickness

Much of our current knowledge of immune complex diseases is based on analyses of experimental models of serum sickness. Immunization of an animal such as a rabbit with a large dose of a foreign protein antigen leads to the formation of antibodies against the antigen (Fig. 19.4). These antibodies bind to the circulating antigen and form immune complexes, which are initially cleared by macrophages in the liver and spleen. As more complexes are formed, some of them are deposited in the walls of blood vessels, where they induce neutrophil-rich inflammation by activating the classical pathway of complement and engaging leukocyte Fc receptors (see Fig. 19.3). The consequence of this inflammatory process depends on the location of the blood vessels. In skin and most organs, the vascular wall inflammation damages endothelial cells lining the vessels, which promotes thrombus formation and thus impairs blood flow to the tissues. The result is ischemic damage and necrosis of those tissues. Capillaries in the renal glomeruli and synovia are sites where plasma is ultrafiltered (to form urine and synovial fluid, respectively) by passing at high pressure through specialized basement membranes, and these locations are among the most common sites of immune complex deposition. Thus, some of the most common clinical and pathologic manifestations are arthritis and nephritis; skin rash is also frequent. The clinical symptoms are usually short-lived, and the lesions heal unless the antigen is injected again. This type of disease is an example of acute serum sickness. A more indolent and prolonged disease, called chronic serum sickness, is produced by multiple injections of small amounts of antigen, which lead to the formation of smaller complexes that are deposited most often in the kidneys, arteries, and lungs.

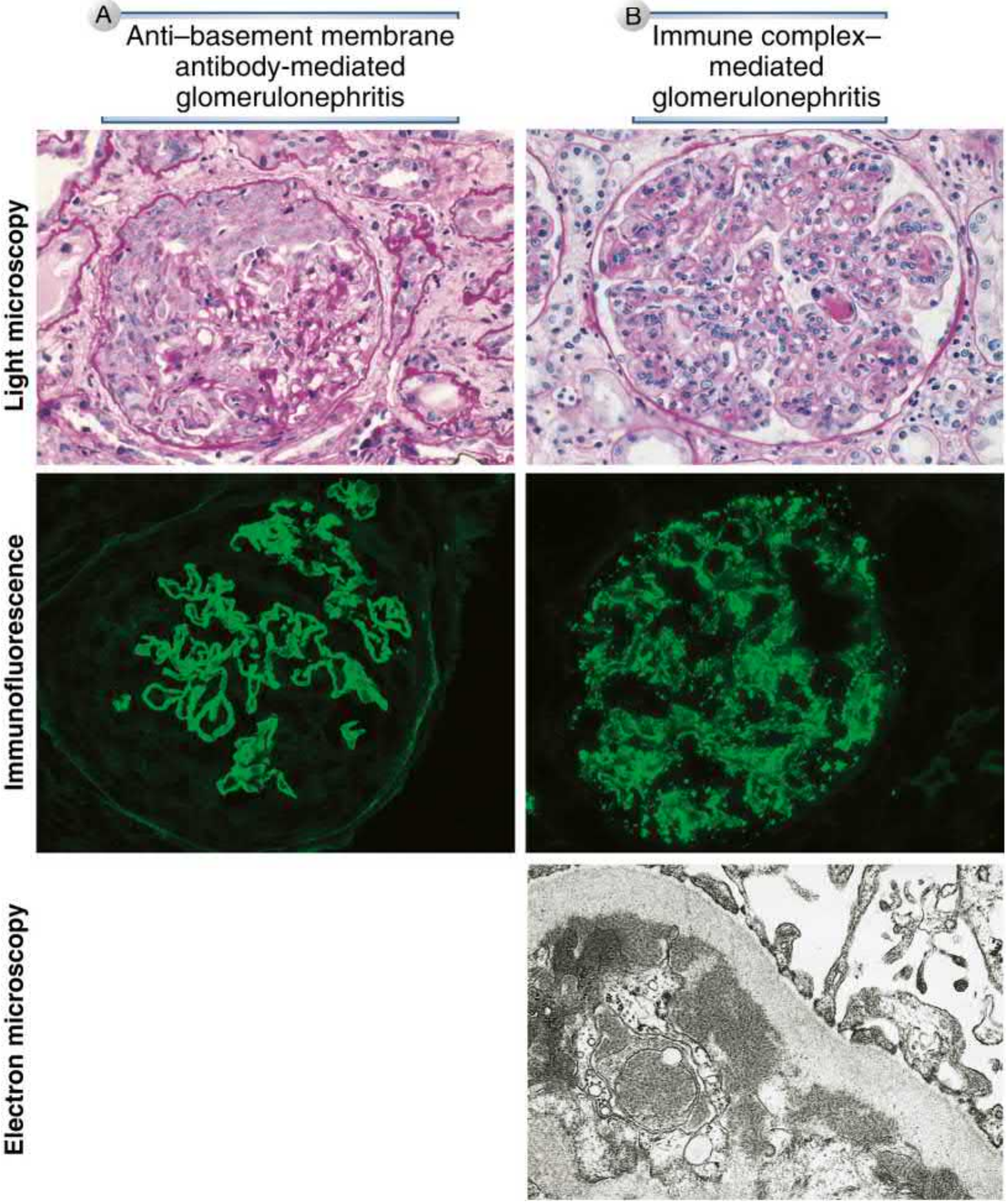

A
Anti–basement membrane antibody-mediated glomerulonephritis
B
Immune complex–mediated glomerulonephritis
Light microscopy
Immunofluorescence
Electron microscopy

FIGURE 19.2 Pathologic features of antibody-mediated glomerulonephritis. **A,** Glomerulonephritis induced by an antibody against the glomerular basement membrane (Goodpasture's syndrome). The light micrograph shows glomerular inflammation and severe damage, and immunofluorescence shows smooth (linear) deposits of antibody along the basement membrane. **B,** Glomerulonephritis induced by the

deposition of immune complexes (systemic lupus erythematosus). The light micrograph shows neutrophilic inflammation, and the immunofluorescence and electron micrograph show coarse (granular) deposits of antigen-antibody complexes along the basement membrane.

Immunofluorescence micrographs are courtesy of Dr. Jean Olson, Department of Pathology, University of California, San Francisco, and the electron micrograph is courtesy of Dr. Helmut Rennke, Department of Pathology, Brigham and Women’s Hospital, Boston, Massachusetts.

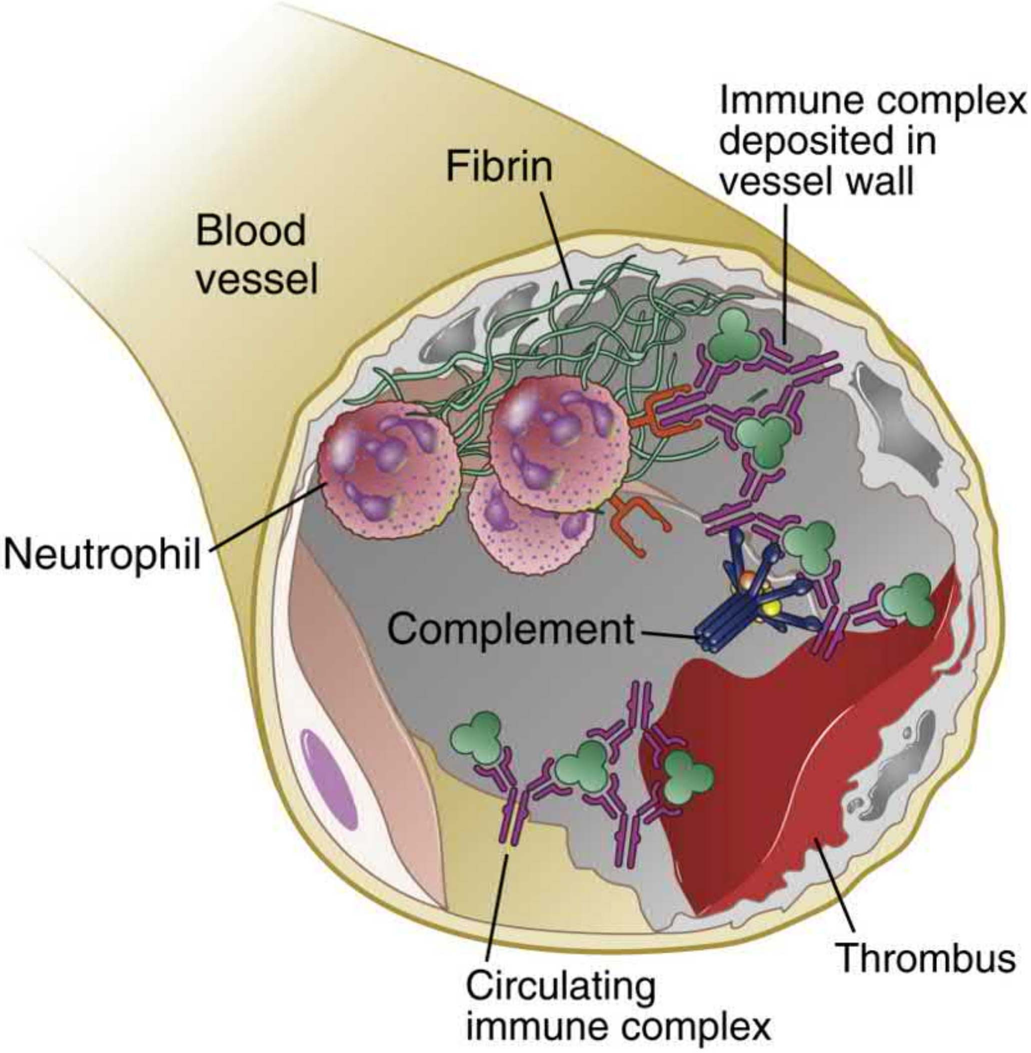

Immune complex deposited in vessel wall
Fibrin
Blood vessel
Neutrophil
Complement
Thrombus
Circulating immune complex

FIGURE 19.3 Immune complex–mediated injury.Circulating immune complexes deposit in vessel walls and induce inflammation (vasculitis) and thrombosis.

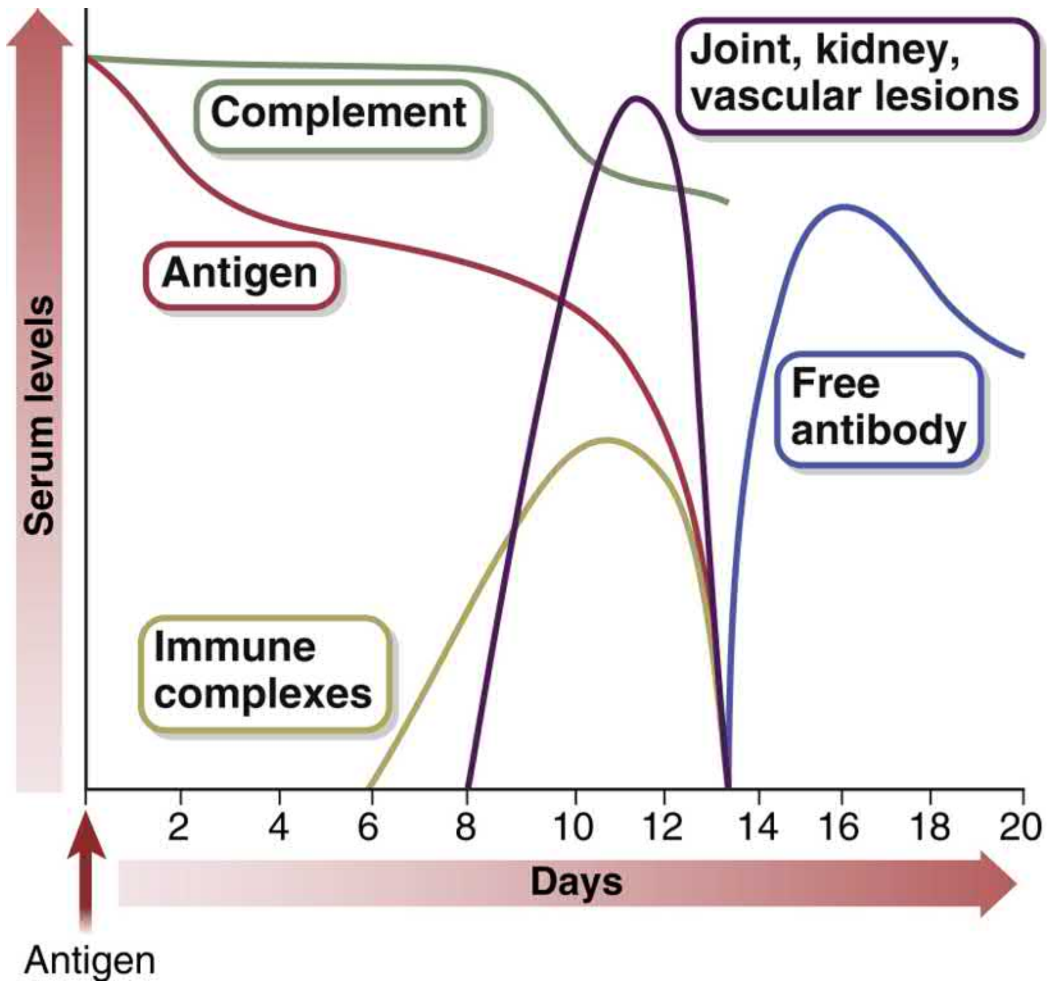


FIGURE 19.4 Sequence of immunologic responses in experimental acute serum sickness. Injection of bovine serum albumin into a rabbit leads to the production of specific antibody and the formation of immune complexes. These complexes are deposited in multiple tissues, activate complement (leading to a decrease in serum complement levels), and cause inflammatory lesions, which resolve as the complexes and the remaining antigen are removed and free antibody (not bound to antigen) appears in the circulation.

Modified from Cochrane CG. Immune complex–mediated tissue injury. In: Cohen S, Ward PA, McCluskey RT, eds. *Mechanisms of immunopathology.* New York, NY: Werbel & Peck; 1979:29–48.

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Arthus Reaction

A localized form of experimental immune complex–mediated vasculitis is called the Arthus reaction. It is induced by subcutaneous injection of an antigen into a previously immunized animal or an animal that has been given an intravenous injection of antibody specific for the antigen. Circulating antibodies rapidly bind to the injected antigen and form immune complexes that are deposited in the walls of small blood vessels at the injection site. This deposition gives rise to a local cutaneous vasculitis, with thrombosis of the affected vessels, leading to tissue necrosis. The clinical relevance of the Arthus reaction is limited; rarely, a subject receiving a booster dose of a vaccine may develop inflammation at the site of injection because of local accumulation of immune complexes, as in an Arthus reaction.

Pathogenesis of Immune Complex–Mediated Diseases

The amount of immune complex deposition in tissues is determined by the nature of the complexes and the characteristics of the blood vessels. Antigen-antibody complexes are produced during most normal immune responses, but they cause disease only when they are produced in excessive amounts, are not efficiently cleared, and deposit in tissues. Small complexes are often not phagocytosed and tend to be deposited in vessels more than large complexes, which are usually cleared by phagocytes. Complexes containing cationic antigens bind avidly to negatively charged components of the basement membranes of blood vessels and kidney glomeruli. Such complexes typically produce severe and long-lasting tissue injury. Although immune complexes are often seen in joints and kidneys, they may be deposited in small vessels in virtually any tissue. Deposits of antibody and complement may be detected in the vessels, and if the antigen is known, it is possible to identify antigen molecules in the deposits as well (Fig. 19.2B). Immune complexes deposited in vessel walls and tissues activate leukocytes and mast cells to secrete cytokines and vasoactive mediators. These mediators may cause more immune complex deposition in vessel walls by increasing vascular permeability and blood flow.

The major mechanism of tissue injury in immune complex diseases is inflammation within the walls of blood vessels that occurs when the antibodies of deposited complexes activate complement and bind to leukocyte Fc receptors. These are the same mechanisms that cause tissue injury in serum sickness, described earlier.

TABLE 19.3

Examples of Human Immune Complex–Mediated Diseases

Disease	**Antigen Involved**	**Clinicopathologic Manifestations**
Systemic lupus erythematosus	DNA, nucleoproteins, others	Nephritis, arthritis, vasculitis
Polyarteritis nodosa	Hepatitis B virus surface antigen (in minority of cases)	Vasculitis
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigens	Nephritis
Serum sickness	Various proteins	Arthritis, vasculitis, nephritis

Many systemic immunologic diseases in humans are caused by the deposition of immune complexes in blood vessels (Table 19.3) **.** Systemic lupus erythematosus (SLE) is an autoimmune disease in which complexes consisting of nuclear antigens and antinuclear antibodies deposit in blood vessels in kidney glomeruli, skin, and many other tissues. In a disorder called polyarteritis nodosa, immune complex–mediated vasculitis involves medium-size muscular arteries. In a small fraction of these patients, the disease is a late complication of viral infection and the complexes are made up of viral antigen (e.g., hepatitis virus antigens) and antibodies. Immune complex deposition is also the mechanism of a disease called poststreptococcal glomerulonephritis, which develops in rare cases after streptococcal infection and is caused by complexes of streptococcal antigen and antibodies depositing in the glomeruli of the kidney (see Fig. 19.2B). In some forms of glomerulonephritis, immune complexes are not detected in the circulation, raising the possibility that the antigens are first planted in the kidney and the complexes form locally.

Diseases Caused by T Lymphocytes

T lymphocytes injure tissues by either producing cytokines that induce inflammation or directly killing target cells (Fig. 19.5) . Inflammatory reactions are elicited mainly by $CD4^+$ T cells of the Th1 and Th17 subsets. In some T cell–mediated disorders, the principal mechanism of tissue injury is killing of cells by $CD8^+$ CTLs. The T cells that cause tissue injury may be autoreactive, or they may be specific for foreign protein antigens that are present in or bound to cells or tissues. T lymphocyte–mediated tissue injury may also accompany strong protective immune responses against persistent microbes, especially intracellular microbes that resist eradication by phagocytes and antibodies.

A role for T cells in causing a particular immunologic disease is suspected largely on the basis of the demonstration of T cells in lesions and the detection of increased levels of cytokines in the blood or tissues that may be derived from T cells. Animal models have been useful for elucidating the pathogenesis of these disorders.

Diseases Caused by Cytokine-Mediated Inflammation

In immune-mediated inflammation, Th1 and Th17 cells secrete cytokines that recruit and activate leukocytes. Recall that inflammation is a major defense reaction of innate immunity (see Chapter 4). When T cells are involved, the inflammation becomes more severe and chronic because T cells induce the production of potent inflammatory mediators for prolonged periods. IL-17, produced by Th17 cells, promotes neutrophil recruitment; interferon-γ (IFN-γ), produced by Th1 cells, activates macrophages; and tumor necrosis factor (TNF) and chemokines, produced by T lymphocytes and cells of innate immunity (such as dendritic cells [DCs] and macrophages), are involved in the recruitment and activation of many types of leukocytes. (Th2 cytokines induce eosinophil-rich allergic inflammation [type I hypersensitivity] and are discussed in Chapter 20.) Although we emphasize $CD4^+$ Th1 and Th17 cells as the sources of cytokines, in lesions many other cells may produce the same cytokines. For instance, in psoriasis, $CD8^+$ T cells also produce IL-17. In some animal models of chronic skin inflammation, a source of IL-17 early in the course of the disease appears to be γδ T cells, and innate lymphoid cells (ILCs) in tissues produce many of the same cytokines as do T cells (see Chapter 4).

Tissue injury results from the products of the recruited and activated neutrophils and macrophages, such as lysosomal enzymes and reactive oxygen species. Cytokines produced by activated lymphocytes and macrophages stimulate more leukocyte recruitment and inflammation, thus propagating the damage (see Chapter 10). Vascular endothelial cells in the lesions may express increased levels of cytokine-regulated surface proteins such as adhesion molecules and class II major histocompatibility complex (MHC) molecules. The inflammation associated with T cell–mediated diseases is typically chronic, but bouts of acute inflammation may be superimposed on a background of chronic inflammation. Delayed-type hypersensitivity (DTH) is an example of such inflammatory reactions and is described later. Chronic inflammatory reactions often produce fibrosis as a result of the secretion of cytokines and growth factors by the macrophages and T cells that activate fibroblasts and stimulate collagen production.

Many organ-specific autoimmune diseases are caused by activation of autoreactive T cells by self antigens, leading to cytokine release and inflammation. This is thought to be the major mechanism underlying rheumatoid arthritis, multiple sclerosis (MS), type 1 diabetes, psoriasis, and other autoimmune diseases (Table 19.4). Some of these diseases are described in more detail at the end of this chapter.

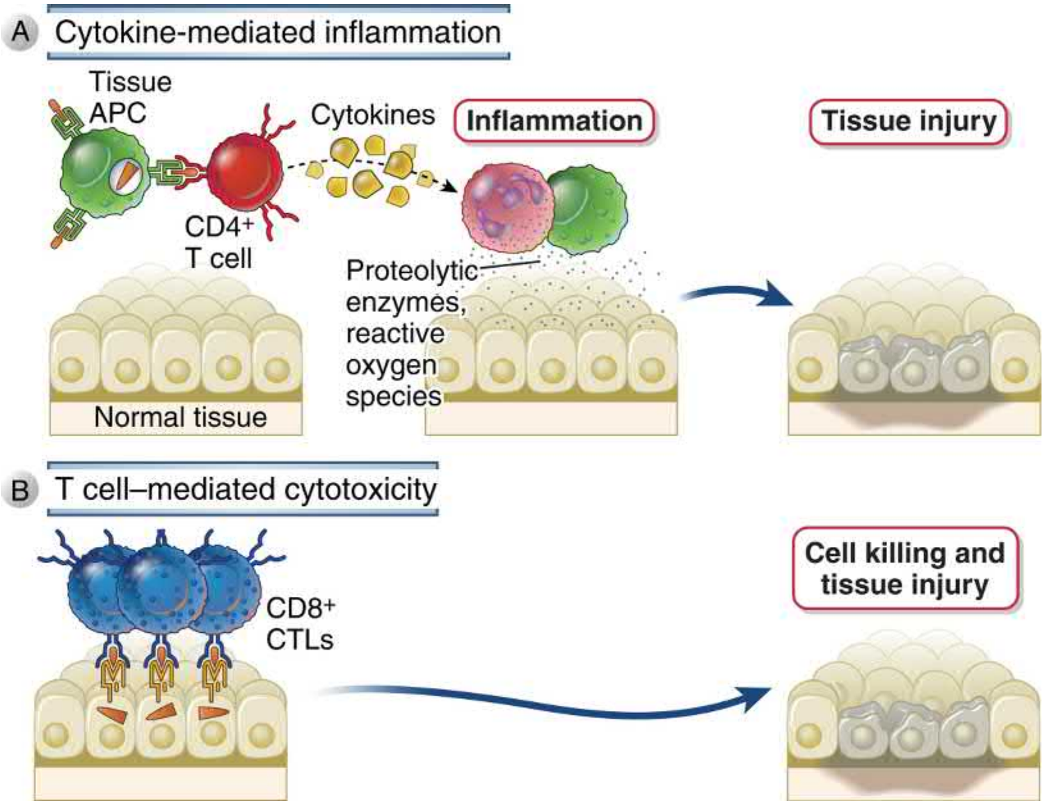
A
Cytokine-mediated inflammation
Tissue APC
CD4+ T cell
Cytokines
Inflammation
Tissue injury
Proteolytic enzymes, reactive oxygen species
Normal tissue
B
T cell–mediated cytotoxicity
CD8+ CTLs
Cell killing and tissue injury

FIGURE 19.5 Mechanisms of T cell–mediated diseases. **A,** In cytokine-mediated inflammatory reactions, $CD4^+$ T cells (and sometimes $CD8^+$ cells, not shown) respond to tissue antigens by secreting cytokines that stimulate inflammation and activate leukocytes, leading to tissue injury. **B,** In some diseases, $CD8^+$ cytotoxic T lymphocytes *(CTLs)* directly kill tissue cells. *APC,* Antigen-presenting cell.

T cell responses specific for microbes and other foreign antigens may also lead to inflammation and tissue injury. Intracellular bacteria such as *Mycobacterium tuberculosis* induce strong T cell and macrophage responses that result in granulomatous inflammation and fibrosis (described later); the inflammation and fibrosis may cause extensive tissue destruction and functional impairment, typically in the lungs. Tuberculosis is a good example of an infectious disease in which tissue injury is mainly due to the host immune response (see Chapter 16). T cell responses against intestinal bacteria are thought to underlie some forms of IBD.

A variety of skin diseases, called contact sensitivity, result from topical exposure to chemicals and environmental antigens. These disorders are caused by inflammatory reactions that are likely triggered by neoantigens formed by the binding of the chemicals to self proteins, including MHC molecules. Both $CD4^+$ and $CD8^+$ T cells may be the source of cytokines in contact sensitivity reactions. Examples of contact sensitivity include rashes induced by poison ivy and poison oak (in which T cells react against self proteins that are modified by plant chemicals called urushiols); by contact with metals (nickel and beryllium) and a variety of chemicals, such as thiuram, which is used in the manufacture of latex gloves; and by therapeutic drugs. Some of these reactions become chronic and clinically are called **eczema** (a clinical term also used to describe a different condition called atopic dermatitis, discussed in Chapter 20).

Delayed-Type Hypersensitivity (DTH)

DTH is an injurious cytokine-mediated inflammatory reaction resulting from the activation of T cells, particularly $CD4^+$ T cells. The reaction is called delayed because it typically develops 24 to 48 hours after antigen challenge in a previously immunized (sensitized) individual, in contrast to immediate hypersensitivity (allergic) reactions, which develop within minutes (described in Chapter 20).

In the classic animal model of DTH, a guinea pig was first immunized by the administration of a protein antigen in adjuvant; this step is called sensitization. About 2 weeks later, the animal was challenged subcutaneously with the same antigen, and the subsequent reaction was analyzed; this step is called the elicitation phase. Humans may be sensitized for DTH reactions by microbial infection, by contact sensitization with chemicals and environmental antigens, or by intradermal or subcutaneous injection of protein antigens (Fig. 19.6). Subsequent exposure to the same antigen (called challenge) elicits the reaction. For example, purified protein derivative (PPD), a protein antigen of *Mycobacterium tuberculosis,* elicits a DTH reaction, called the tuberculin reaction, when it is injected into individuals who have been exposed to *M. tuberculosis*. A positive tuberculin skin test response is a widely used clinical indicator of previous or active tuberculosis infection.

The characteristic response of DTH evolves over 24 to 48 hours. About 4 hours after the injection of an antigen in a sensitized individual, neutrophils accumulate around the postcapillary venules at the injection site. By about 12 hours, the injection site becomes infiltrated by T cells and blood monocytes, also organized in a perivenular distribution (Fig. 19.7). The endothelial cells lining these venules become enlarged and show increased organelles, and the vessels leak plasma macromolecules. Fibrinogen escapes from the blood vessels into the surrounding tissues, where it is converted into fibrin. The deposition of fibrin, edema, and the accumulation of T cells and monocytes within the extravascular tissue space around the injection site cause the tissue to swell and become firm (indurated). Induration, a diagnostic feature of DTH, is detectable by about 18 hours after the injection of antigen and is maximal by 24 to 48 hours. In clinical practice, loss of DTH responses to universally encountered antigens (e.g., *Candida* antigens) is an indication of deficient T cell function, a condition known as **anergy.** (This general loss of immune responsiveness is different from lymphocyte anergy, a mechanism for maintaining tolerance to specific antigens, discussed in Chapter 15.)

TABLE 19.4

T Cell–Mediated Diseases

Disease	Specificity of Pathogenic T Cells	Principal Mechanisms of Tissue Injury
Rheumatoid arthritis	Collagen? Citrullinated self proteins?	Inflammation mediated by Th1 and Th17 cytokines. Role of antibodies and immune complexes?
Multiple sclerosis	Protein antigens in myelin (e.g., myelin basic protein)	Inflammation mediated by Th1 and Th17 cytokines; myelin destruction by activated macrophages
Type 1 diabetes mellitus	Antigens of pancreatic islet β cells (insulin, glutamic acid decarboxylase, others)	T cell–mediated inflammation; destruction of islet cells by CTLs
Inflammatory bowel disease	Enteric bacteria. Self antigens?	Inflammation mediated by Th1 and Th17 cytokines
Psoriasis	Unknown skin antigens	Inflammation mediated by T cell–derived cytokines

Examples of human T cell–mediated diseases are listed. In many cases, the specificity of the T cells and the mechanisms of tissue injury are inferred on the basis of the similarity with experimental animal models of the diseases. The roles of Th1 and Th17 cells have been inferred from experimental models and the presence of subset-specific cytokines in human lesions. The cytokines may be produced by cells other than $CD4^+$ T lymphocytes. Ongoing clinical trials targeting these cytokines may provide new information about the contributions of the cytokines in different diseases.

CTLs, Cytotoxic T lymphocytes.

Although DTH has traditionally been considered a Th1-mediated injurious reaction, other T cells may contribute to the inflammation. In some DTH lesions, neutrophils are prominent, suggesting the involvement of Th17 cells. In infections by some helminthic parasites, reactions against the parasite eggs elicit DTH with a strong component of eosinophils. In these cases, a role for Th2 cytokines has been demonstrated. $CD8^+$ T cells also produce IFN-γ and contribute to DTH reactions, especially in the skin.

Chronic DTH reactions and fibrosis can develop if a Th1 response to an infection activates macrophages but fails to eliminate phagocytosed microbes. With some infections, the reaction produces nodules of inflammatory tissue called granulomas (Fig. 19.8A). Chronic DTH, as exemplified by granulomatous inflammation, is caused by prolonged cytokine signals (Fig. 19.8B). In such reactions, the activated T cells and macrophages continue to produce cytokines and growth factors, which amplify the reactions of both cell types and progressively modify the local tissue environment by activating fibroblasts that lay down collagen. The result is a cycle of tissue injury and chronic inflammation followed by replacement with connective tissue (fibrosis). In chronic DTH reactions, activated macrophages also respond to persistent cytokine signals by increasing cytoplasm and cytoplasmic organelles. Histologically, these macrophages may resemble skin epithelial cells, because of which they are sometimes called epithelioid cells. Activated macrophages may fuse to form multinucleate giant cells. Granulomatous inflammation is an attempt to contain the infection but is also the cause of significant tissue injury and functional impairment. This type of inflammation is a characteristic response to some persistent microbes, such as *M. tuberculosis,* and some fungi. Much of the respiratory difficulty associated with tuberculosis or chronic fungal infection of the lungs is caused by replacement of normal lung tissue with fibrotic tissue and is not directly attributable to the microbes.

Diseases Caused by Cytotoxic T Lymphocytes

CTL responses to viral infection can lead to tissue injury by killing infected cells, even if the virus itself has little cytopathic effect. The principal physiologic function of CTLs is to eliminate intracellular microbes, primarily viruses, by killing infected cells. Some viruses directly injure infected cells and are said to be cytopathic, whereas others are not. Because CTLs cannot distinguish between cytopathic and noncytopathic viruses, they kill virus-infected cells regardless of whether the infection itself is harmful to the host. Examples of viral infections in which the lesions are mainly due to the host CTL response and not the virus itself include lymphocytic choriomeningitis in mice and certain forms of viral hepatitis in humans (see Chapter 16).

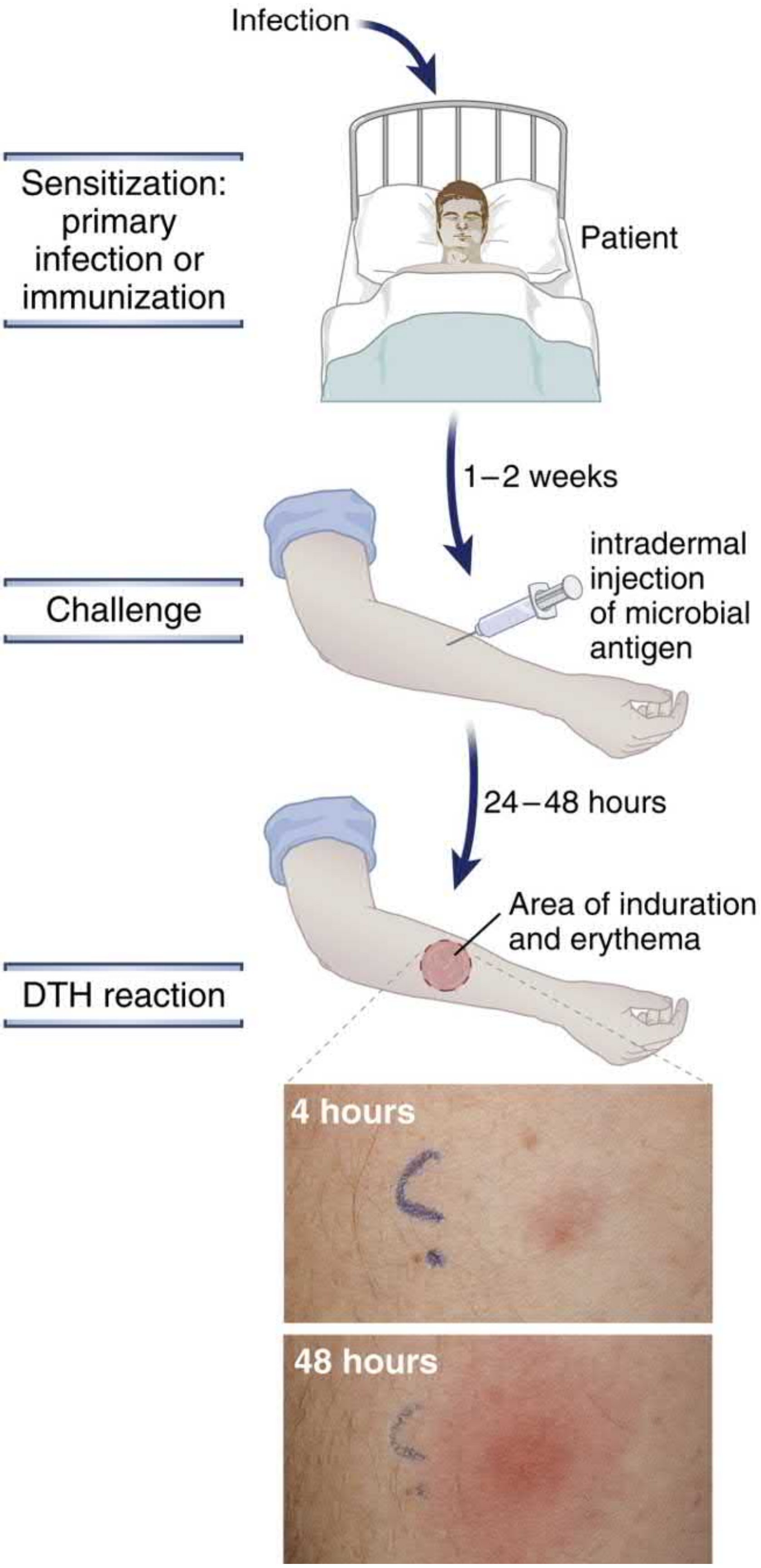
Infection
Sensitization: primary infection or immunization
Patient
1–2 weeks
Challenge
intradermal injection of microbial antigen
24–48 hours
DTH reaction
Area of induration and erythema
4 hours
48 hours

FIGURE 19.6 Delayed-type hypersensitivity reaction. Infection or immunization (vaccination) sensitizes an individual, and subsequent challenge with an antigen from the infectious agent elicits a delayed-type hypersensitivity *(DTH)* reaction. The reaction is manifested by induration with redness and swelling at the site of the challenge, which peaks at approximately 48 hours.

Courtesy Dr. J. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.

CTLs may contribute to tissue injury in autoimmune disorders in which destruction of particular host cells is a prominent component, such as type 1 diabetes, in which insulin-producing β cells in pancreatic islets are destroyed. CTLs also cause injury to organ allografts during rejection responses (see Chapter 17).

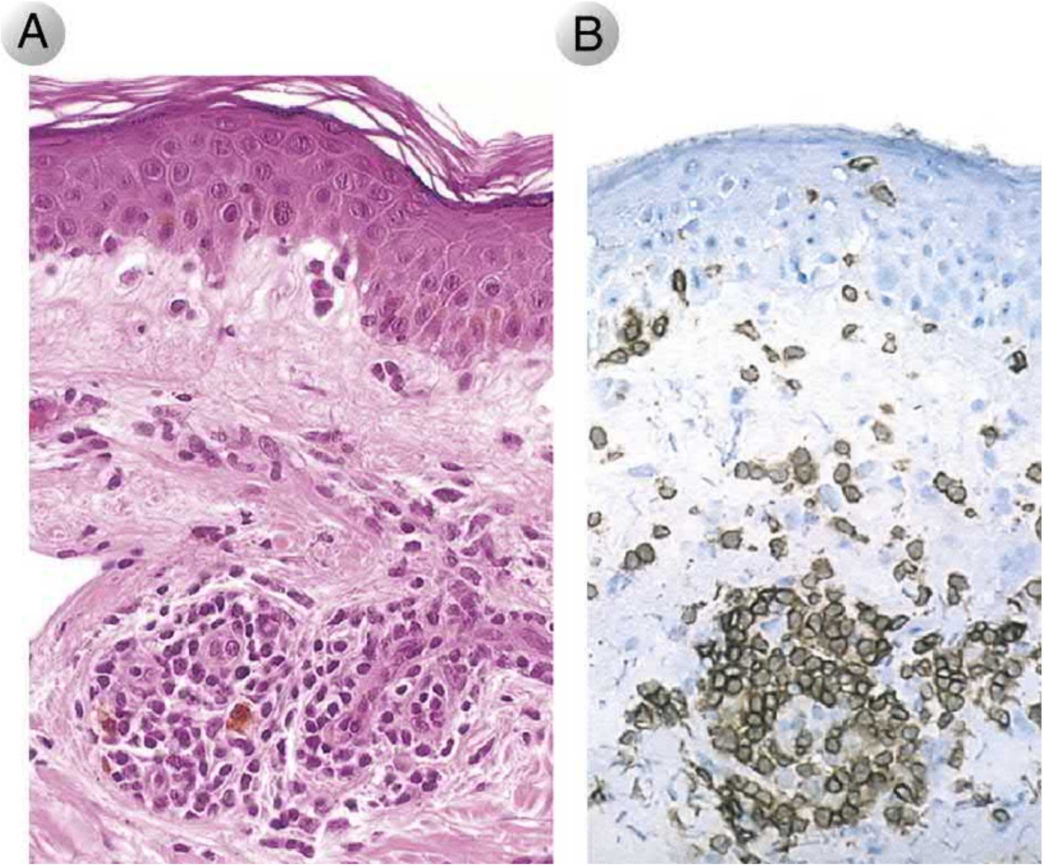

FIGURE 19.7 Morphology of a delayed-type hypersensitivity reaction. **A,** Histopathologic examination of the reaction in skin illustrated in Fig. 19.6 shows perivascular mononuclear cell infiltrates in the dermis. At higher magnification (not shown), the infiltrate is seen to consist of activated lymphocytes and macrophages surrounding small blood vessels in which the endothelial cells are also activated. **B,** Immunohistochemical staining demonstrates the presence of many $CD4^+$ T lymphocytes.

Courtesy Dr. J. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, California.

Therapeutic Approaches for Immunologic Diseases

One of the most impressive accomplishments of immunology has been the development of novel therapies for immunologic diseases based on the understanding of fundamental mechanisms of these disorders (Fig. 19.9). The therapies can be divided into several broad groups.

Broadly Acting Antiinflammatory Agents

The mainstay of therapy for hypersensitivity diseases for many years has been antiinflammatory drugs, particularly corticosteroids. Such drugs inhibit the secretion of cytokines and other mediators of inflammation and thus reduce the inflammation associated with pathologic immune responses. Nonsteroidal antiinflammatory drugs are commonly used to reduce milder inflammatory reactions.

Cytokine Antagonists

A large number of cytokines and their receptors involved in inflammation are being targeted by specific antagonists for the treatment of chronic T cell–mediated inflammatory diseases (Table 19.5). The first clinically succcessful cytokine antagonists targeted TNF and included a soluble form of the TNF receptor and anti-TNF antibodies, which bind to and neutralize TNF. These agents are of great benefit in many patients with rheumatoid (RA), Crohn's disease, and psoriasis. Antibodies to the IL-6 receptor also have been successfully used in some forms of arthritis. Antagonists of other proinflammatory cytokines and their receptors, including IL-1, IL-12, IL-17, and the receptors for IL-12, IL-17, and IL-23, are now approved for various inflammatory diseases, and many others are in clinical trials. Antibodies against Th2 cytokines or their receptors are approved for treating allergic diseases (see Chapter 20). In addition to these biologic agents, small molecule inhibitors of Janus kinases (JAKs) (important intracellular signaling mediators of a variety of cytokine receptors; see Chapter 7) are also approved to inhibit cytokine actions in RA, and inhibitors of other kinases (such as the B cell signaling molecule BTK) are approved for antibody-mediated diseases (e.g., RA and SLE).

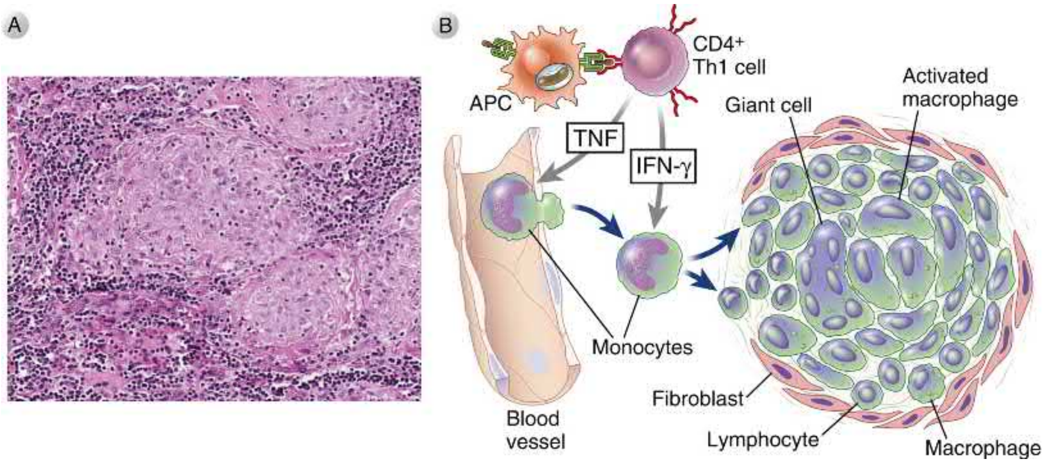
A
B
APC
CD4+
Th1 cell
TNF
IFN-γ
Giant cell
Activated
macrophage
Monocytes
Blood
vessel
Fibroblast
Lymphocyte
Macrophage

FIGURE 19.8 Granulomatous inflammation. **A,** Lymph node from a patient with tuberculosis containing granulomas with activated macrophages, multinucleate giant cells, and lymphocytes. In some granulomas, there may be a central area of necrosis (not shown). Immunohistochemical studies would identify the lymphocytes as T cells. **B,** Mechanisms of granuloma formation. Cytokines are involved in the generation of Th1 cells, activation of macrophages, and recruitment of leukocytes. Prolonged reactions of this type lead to the formation of granulomas. *APC,* Antigen-presenting cell; *IFN-γ,* interferon-γ; *TNF,* tumor necrosis factor.

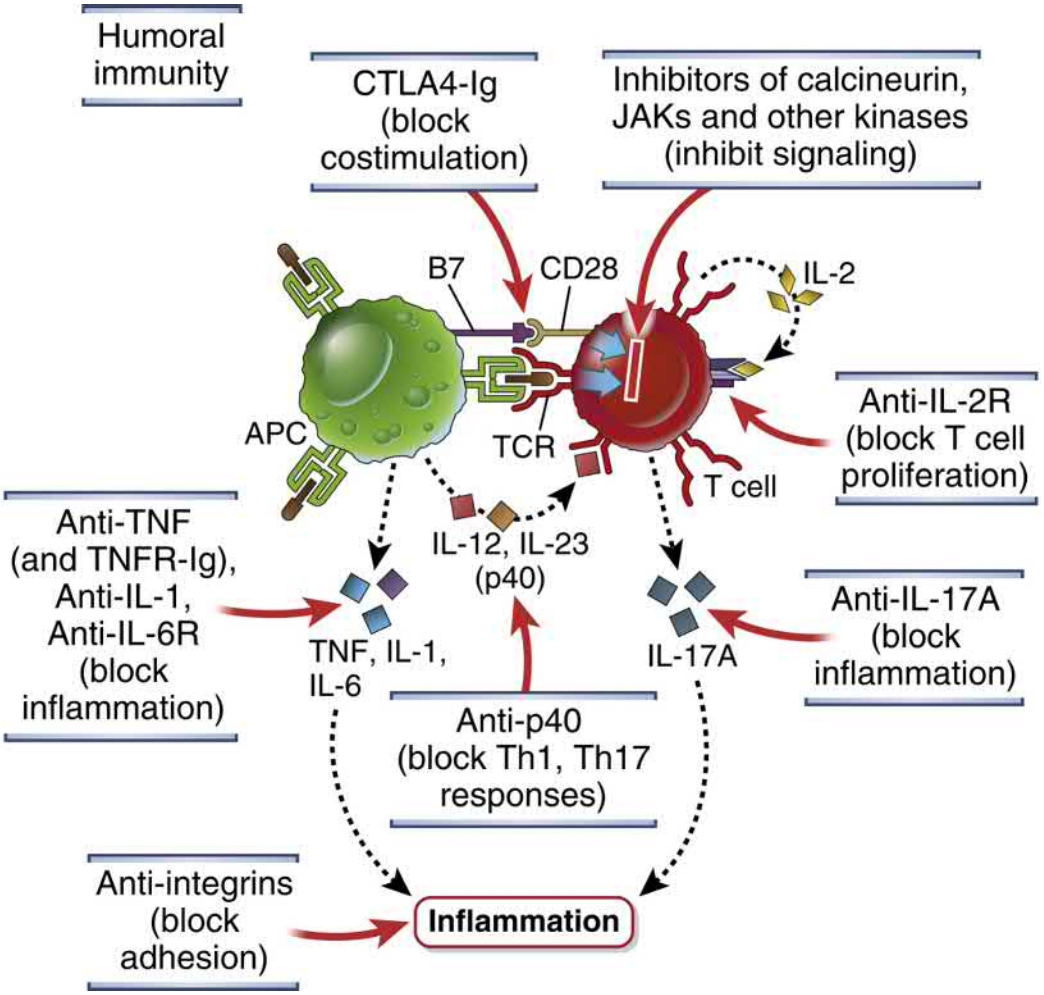
Humoral immunity
CTLA4-Ig (block costimulation)
Inhibitors of calcineurin, JAKs and other kinases (inhibit signaling)
B7
CD28
IL-2
APC
TCR
T cell
Anti-IL-2R (block T cell proliferation)
Anti-TNF (and TNFR-Ig), Anti-IL-1, Anti-IL-6R (block inflammation)
IL-12, IL-23 (p40)
TNF, IL-1, IL-6
IL-17A
Anti-IL-17A (block inflammation)
Anti-p40 (block Th1, Th17 responses)
Anti-integrins (block adhesion)
Inflammation

FIGURE 19.9 Biologic therapies for inflammatory diseases targeting T cell responses and inflammation. Illustrated are the sites of action of some therapeutic agents that block different components of immune and inflammatory responses. Many of these agents target cytokines and their receptors. B cell depletion by anti-CD20 may also reduce pathologic T cell responses (not shown). *APC,* Antigen-presenting cell; *CTLA-4,* cytotoxic T lymphocyte antigen 4; *IL,* interleukin; *JAKs,* Janus kinases; *TCR,* T cell receptor; *TNF,* tumor necrosis factor; *TNFR-Ig,* TNF receptor-immunoglobulin.

TABLE 19.5

Examples of Cytokine Antagonists in Clinical Use or Trials

Cytokine or Receptor Targeted	Predicted Biologic Effects of Antagonist	Clinical Indications
TNF	Inhibits leukocyte migration into sites of inflammation	Rheumatoid arthritis, psoriasis, inflammatory bowel disease
IL-1	Inhibits leukocyte migration into sites of inflammation	Rare autoinflammatory syndromes, severe gout, rheumatoid arthritis
IL-6 receptor	Inhibits inflammation, antibody responses?	Juvenile idiopathic arthritis, rheumatoid arthritis
IL-17	Inhibits leukocyte recruitment into sites of inflammation	Psoriasis
IL-17 receptor	Inhibits leukocyte recruitment into sites of inflammation	Psoriasis
p40 chain of IL-12 and IL-23	Inhibits Th1 and Th17 development	Inflammatory bowel disease, psoriasis

Cytokine or Receptor Targeted	Predicted Biologic Effects of Antagonist	Clinical Indications
IL-2 receptor (CD25)	Inhibits IL-2–mediated T cell proliferation	Acute graft rejection
BAFF	Reduces survival of B lymphocytes	Systemic lupus erythematosus

The table lists examples of antagonists against cytokines (antibodies or soluble receptors) that are approved for clinical use or in trials. Monoclonal antibodies specific for each of the listed targets are in clinical use; soluble TNF receptor and IL-1 receptor antagonists are used as well.

BAFF, B cell activating factor; *IL*, interleukin; *TNF*, tumor necrosis factor.

Depletion of Cells and Antibodies

Monoclonal antibodies that deplete all lymphoid cells, only B cells, or only T cells are used to treat inflammatory diseases. In Chapter 5, we listed some of the depleting antibodies in clinical practice (see Table 5.3). A recent development is the successful use of anti-CD20 antibody (rituximab), which depletes only B cells, to treat diseases that were thought to be caused primarily by T cell–mediated inflammation. This treatment has shown efficacy in patients with RA and MS. The effectiveness of anti-CD20 may be related to a role of B cells as antigen-presenting cells (APCs) for T cell responses, especially for the generation and maintenance of memory T cells. Plasmapheresis has been used to eliminate circulating autoantibodies and immune complexes.

Other Biologic Agents

CTLA-4-Ig, a fusion protein of the extracellular domain of CTLA-4 and an IgG Fc region, blocks B7 costimulators (see Chapter 9) and is approved for treatment of RA and graft rejection. Antibodies against integrins have been used to inhibit leukocyte migration into tissues, particularly the central nervous system (CNS) in MS patients (anti-VLA-4) and the intestines in IBD patients (anti-$\alpha_4\beta_7$).

Intravenous IgG

Intravenous Ig (IVIG) is pooled IgG from healthy donors administered intravenously. It has beneficial effects in some autoimmune diseases, such as autoimmune thrombocytopenia and hemolytic anemia. It is not clear how this agent, which contains IgG of many unknown specificities, inhibits hypersensitivity reactions. One possibility is that the IgG binds to the inhibitory Fc receptor (FcγRIIB) on B lymphocytes (see Chapter 12) and DCs and thus attenuates autoantibody production and inflammatory responses. IVIG may also compete with pathogenic antibodies for binding to the neonatal Fc receptor (FcRn), which functions in adults to protect antibodies from catabolism (see Chapter 5), resulting in reduced half-lives of the pathogenic antibodies.

Tolerance-Inducing Therapies

There are ongoing attempts at more specific treatments for adaptive immune mediated inflammatory diseases, such as inducing tolerance in disease-producing T cells. MS and type 1 diabetes are two diseases in which the target antigens have been defined; in both disorders, clinical trials are underway in which the antigens (peptides of myelin basic protein [MBP] and insulin, respectively) are administered to patients in ways that inhibit lymphocytes specific for the antigens. A risk with many treatments that block various components of the immune system is that these will interfere with the normal function of the immune system in combating microbes and thus make individuals susceptible to infections. Antigen-specific tolerance avoids this problem by selectively targeting the disease-causing lymphocytes.

There is also interest in exploiting our knowledge of regulatory T cells (Tregs) to treat inflammatory diseases. Numerous clinical trials are ongoing to purify patients' Tregs, expand and activate them in culture, and transfer them back to the patients. Another approach is to treat patients with low doses of IL-2, which is expected to activate and maintain Tregs more than effector cells, or IL-2 that is mutated to bind preferentially to CD25, the IL-2 receptor chain that is expressed at constant and high levels in Tregs.

Selected Immunologic Diseases: Pathogenesis and Therapeutic Strategies

In the following section, we will describe the pathogenesis of selected diseases that are caused by antibodies and T cells and novel therapies for these diseases. The goal of this discussion is not to present clinical details but to focus on how diseases illustrate the principles underlying abnormal immune reactions.

Systemic Lupus Erythematosus (SLE): The Prototypic Immune Complex–Mediated Disease

SLE is a chronic, remitting and relapsing, multisystem autoimmune disease that affects predominantly women, with an incidence in the United States of 1 in 700 among women 20 to 60 years of age (about 1 in 250 among black women) and a female-to-male ratio of 10:1. SLE is considered the classic human immune complex disease. The principal clinical manifestations are rashes, arthritis, and glomerulonephritis, but hemolytic anemia, thrombocytopenia, and neuropsychiatric disorders are also common. Many different autoantibodies are found in patients with SLE. The most frequent are antinuclear, particularly anti-DNA, antibodies; others include antibodies against ribonucleoproteins, histones, and nucleolar antigens. Immune complexes formed from these autoantibodies and their specific antigens deposit in small arteries and capillaries throughout the body and are responsible for glomerulonephritis, arthritis, and vasculitis. Hemolytic anemia and thrombocytopenia are caused by autoantibodies against erythrocytes and platelets, respectively. The principal diagnostic test for the disease is the presence of antinuclear antibodies; antibodies against double-stranded native DNA are specific for SLE.

Pathogenesis of Systemic Lupus Erythematosus

In SLE, genetic and environmental factors contribute to a breakdown of tolerance in self-reactive B and T lymphocytes. Among the genetic factors is the inheritance of particular HLA (human leukocyte antigen) alleles. The odds ratio (relative risk) for individuals with HLA-DR2 or HLA-DR3 is 2 to 3, and if both haplotypes are present, the odds ratio is about 5. Genetic deficiencies of classical pathway complement proteins, especially C1q, C2, or C4, are seen in about 5% of patients with SLE. The complement deficiencies may result in defective clearance of immune complexes and apoptotic cells and failure of B cell tolerance. A polymorphism in the inhibitory Fc receptor FcγRIIB has been described in some patients; this may contribute to inadequate control of B cell activation or a failure to attenuate inflammatory responses in innate immune cells. Many other genes have been detected by genome-wide association studies, and the roles of some of these, such as the phosphatase PTPN22, have been discused in Chapter 15. Environmental factors include exposure to ultraviolet (UV) light, which is postulated to lead to the apoptotic death of skin cells and release of nuclear antigens.

Two observations have led to new hypotheses of the pathogenesis of SLE. First, studies in patients have revealed that blood cells show a striking molecular signature (pattern of gene expression) that indicates exposure to IFN-α, a type I interferon that is produced mainly by plasmacytoid DCs. Some studies have shown that plasmacytoid DCs from SLE patients produce abnormally large amounts of IFN-α. Second, studies in animal models have shown that Toll-like receptors (TLRs) that recognize DNA and RNA, notably the DNA-recognizing TLR9 and the RNA-recognizing TLR7, play a role in the activation of B cells specific for self nuclear antigens. On the basis of these studies, a model for the pathogenesis of SLE has been proposed (Fig. 19.10). According to this model, UV irradiation and other environmental insults lead to the apoptosis of cells. Inadequate clearance of the nuclei of these cells, in part because of defects in clearance mechanisms such as complement proteins and nucleases such as TREX1, results in a large burden of nuclear antigens. Polymorphisms in various susceptibility genes for lupus lead to a defective ability to maintain self-tolerance in B and T lymphocytes, because of which self-reactive lymphocytes remain functional. Failure of B cell tolerance may be due to defects in receptor editing or in deletion of immature B cells in the bone marrow or in peripheral tolerance. Self-reactive B cells that are not rendered tolerant are stimulated by the self nuclear antigens, and antibodies are produced against the antigens. Complexes of the antigens and antibodies bind to Fc receptors on DCs and to the antigen receptor on B cells and may be internalized into endosomes. The nucleic acid components engage endosomal TLRs and stimulate B cells to produce more autoantibodies and activate DCs, particularly plasmacytoid DCs, to produce IFN-α, which further enhances the immune response and may cause more apoptosis. The net result is a cycle of antigen release and immune activation that leads to the production of high-affinity autoantibodies.

New Therapies for Systemic Lupus Erythematosus

The recent advances in our understanding of SLE are leading to novel therapeutic attempts, but success has proven elusive. There has been great interest in depleting B cells by use of an antibody against the B cell surface protein CD20, but clinical trials using anti-CD20 have had little success. An antibody that blocks the B cell growth factor, B cell–activating factor (BAFF), is now approved for the treatment of SLE but seems to have only modest efficacy. Additional approaches that are being tried are to combine B cell depletion with depletion of long-lived plasma cells using proteasome inhibitors (which lead to the accumulation of misfolded proteins and ultimately cell death) and to activate Tregs by treating patients with low-dose IL-2 (see Chapter 15). Despite the involvement of IFN-α in the disease, clinical trials to test the efficacy of antibodies against IFN-α or its receptor have not been successful.

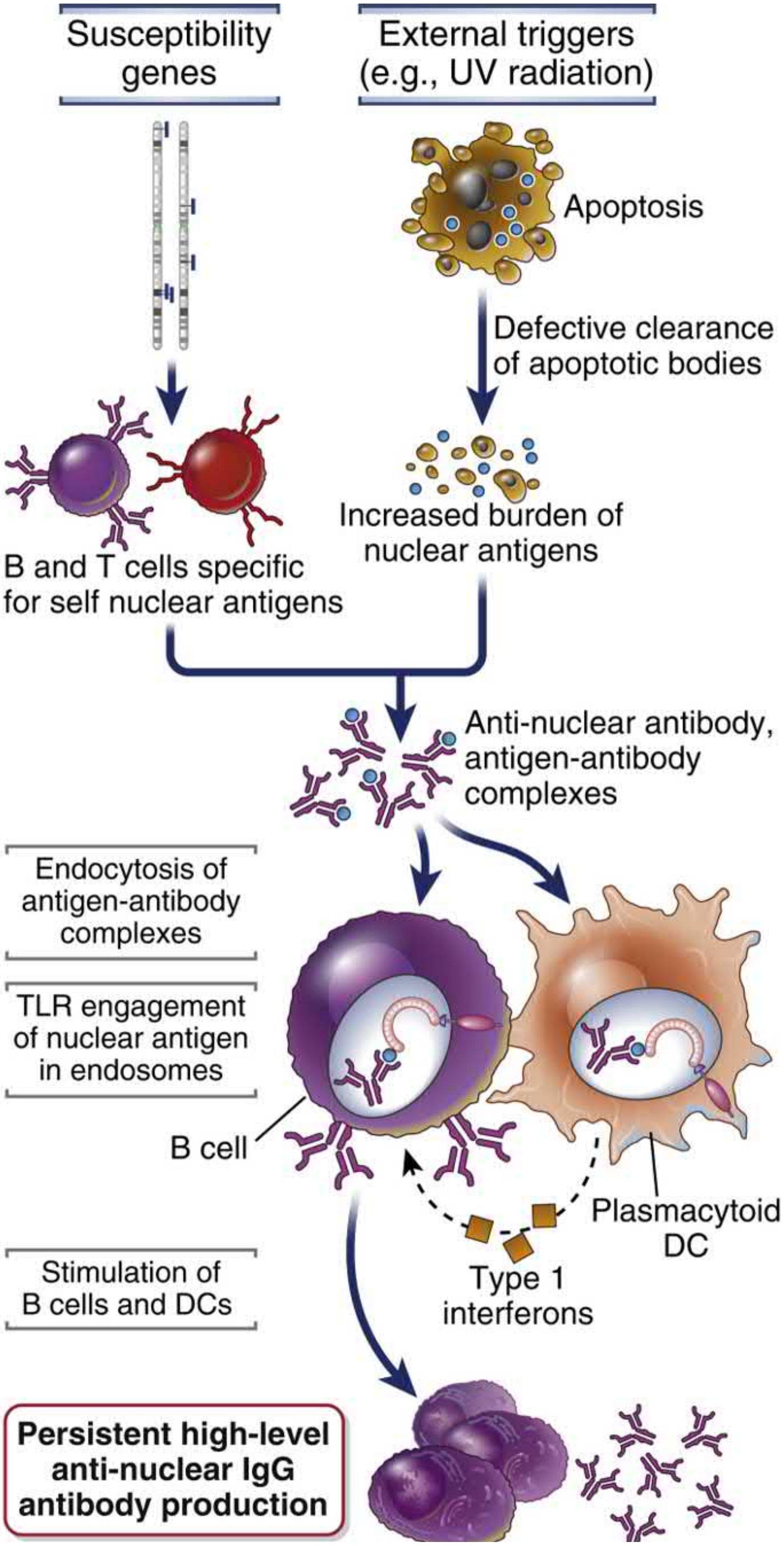
Susceptibility genes
External triggers (e.g., UV radiation)
Apoptosis
Defective clearance of apoptotic bodies
B and T cells specific for self nuclear antigens
Increased burden of nuclear antigens
Anti-nuclear antibody, antigen-antibody complexes
Endocytosis of antigen-antibody complexes
TLR engagement of nuclear antigen in endosomes
B cell
Plasmacytoid DC
Type 1 interferons
Stimulation of B cells and DCs
Persistent high-level anti-nuclear IgG antibody production

FIGURE 19.10 A model for the pathogenesis of systemic lupus erythematosus.In this hypothetical model, various susceptibility genes interfere with the maintenance of self-tolerance and external triggers lead to persistence of nuclear antigens. The result is an antibody response against self nuclear antigens, which is amplified by the Toll-like receptor *(TLR)*-dependent activation of dendritic cells *(DCs)* and B cells by nucleic acids, and the production of type 1 interferons. *IgG,* Immunoglobulin G; *UV,* ultraviolet.

Rheumatoid Arthritis (RA)

RA is an inflammatory disease involving small and large joints of the extremities, including fingers and toes, wrists, shoulders, knees, and ankles. The disease is characterized by inflammation of the synovium associated with destruction of the joint cartilage and bone, with a morphologic picture indicative of a local immune response. Both cell-mediated and humoral immune responses may contribute to development of synovitis. $CD4^+$ Th1 and Th17 cells, activated B lymphocytes, plasma cells, and macrophages as well as other inflammatory cells, are found in the inflamed synovium, and in severe cases, well-formed lymphoid follicles with germinal centers (so-called tertiary lymphoid organs) may be present. Numerous cytokines, including IL-1, IL-8, TNF, IL-6, IL-17, and IFN-γ, have been detected in the synovial (joint) fluid. Cytokines are thought to recruit leukocytes whose products cause tissue injury and also to activate resident synovial cells to produce proteolytic enzymes, such as collagenase, that mediate destruction of the cartilage, ligaments, and tendons of the joints. Increased osteoclast activity in the joints contributes to the bone destruction in RA, and this may be caused by the production of the TNF family cytokine RANK (receptor activator of nuclear factor κB) ligand by activated T cells. RANK ligand binds to RANK, a member of the TNF receptor family that is expressed on osteoclast precursors and induces their differentiation and activation. Systemic complications of RA include vasculitis, presumably caused by immune complexes, and lung injury with fibrosis.

Although much of the emphasis in studies of RA has been on the role of T cells, antibodies may also contribute to the joint destruction. Activated B cells and plasma cells are often present in the synovia of affected joints. Patients frequently have circulating IgM or IgG antibodies that react with the Fc (and rarely Fab) portions of their own IgG molecules. These autoantibodies are called rheumatoid factors, and their presence is used as a diagnostic test for RA. Another type of antibody that has been detected in over half of patients is specific for citrullinated proteins. (The antibodies are called anti-citrullinated protein antibodies, or ACPAs, because they are assayed by binding to citrullinated peptides.) These chemically altered antigens are derived from proteins such as vimentin and fibrinogen, among others, that are modified in an inflammatory environment by the enzymatic conversion of arginine residues to citrulline. About 60% to 80% of RA patients have rheumatoid factor and/or ACPAs and are said to have seropositive RA, which tends to be more severe than non-seropositive RA. Many asymptomatic seropositive individuals have been studied and observed to gradually develop seropositive RA. Both types of antibodies are diagnostic markers and may be involved in the formation of pathogenic immune complexes.

Pathogenesis of Rheumatoid Arthritis

Like other autoimmune diseases, RA is a complex disorder in which genetic and environmental factors contribute to the breakdown of tolerance to self antigens. The specificity of the pathogenic T and B cells remains unclear, although both B and T cells that recognize citrullinated proteins have been identified. Susceptibility to RA is linked to the HLA-DR4 haplotype and to a few other HLA-DR alleles, all of which share a five-residue segment (called the shared epitope) in the peptide-binding groove. Recent genome-wide association studies have revealed a large number of genetic polymorphisms associated with RA, including the gene encoding a tyrosine phosphatase, PTPN22, discussed in Chapter 15.

The identification of ACPAs has led to new ideas about the pathogenesis of RA (Fig. 19.11). Some of the earliest ACPAs tend to be of the IgA isotype, so it is postulated that this disease in initiated at mucosal sites, including the respiratory tract. According to one model, environmental insults, such as smoking and some infections, induce the citrullination of self proteins, leading to the creation of new antigenic epitopes. Because these chemically modified epitopes are neoantigens that are not present normally, there may not be tolerance to these antigens. Individuals who have the HLA alleles that are capable of presenting these epitopes may mount T cell and antibody responses against the proteins. If these modified self proteins are also present in joints, the T cells and antibodies attack the joints. Th17 and perhaps Th1 cells secrete cytokines that recruit leukocytes into the joint and activate synovial cells to produce collagenases and other enzymes. The net result is the progressive destruction of cartilage and bone. The chronic immune responses in the joints may lead to formation of tertiary lymphoid organs in the synovium, and these may maintain and propagate the local immune reaction.

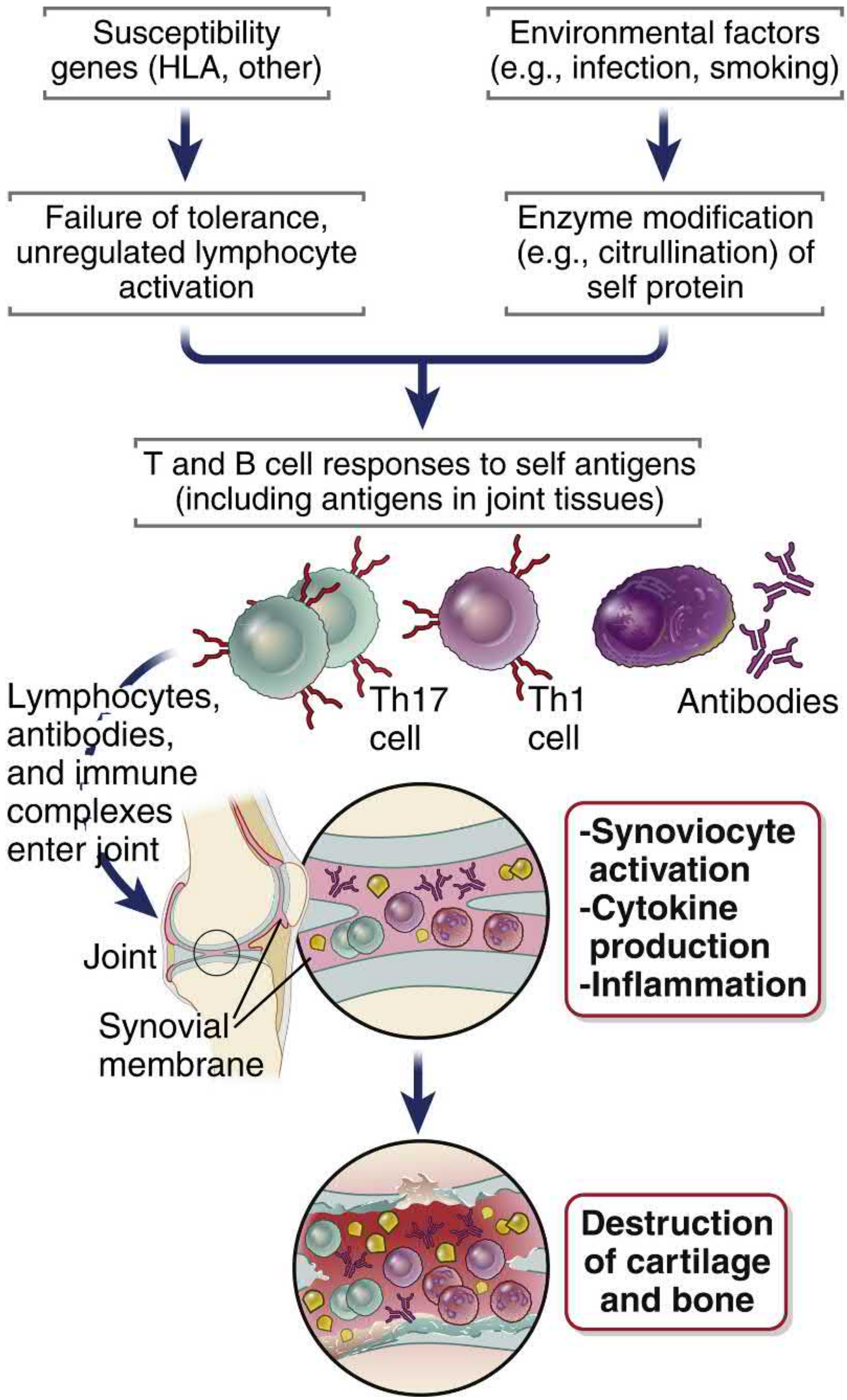
Susceptibility genes (HLA, other)
Environmental factors (e.g., infection, smoking)
Failure of tolerance, unregulated lymphocyte activation
Enzyme modification (e.g., citrullination) of self protein
T and B cell responses to self antigens (including antigens in joint tissues)
Th17 cell
Th1 cell
Antibodies
Lymphocytes, antibodies, and immune complexes enter joint
Joint
Synovial membrane
-Synoviocyte activation
-Cytokine production
-Inflammation
Destruction of cartilage and bone

FIGURE 19.11 A model for the pathogenesis of rheumatoid arthritis.According to this model, citrullinated proteins induced by environmental stimuli elicit T cell and antibody responses in genetically susceptible individuals. The T cells and antibodies enter joints, respond to the self proteins, and cause tissue injury mainly by cytokine secretion and perhaps also by antibody-dependent effector mechanisms. Protein modifications other than citrullination may lead to the same result. *HLA,* Human leukocyte antigen.

New Therapies for Rheumatoid Arthritis

The realization of the central role of T cells and cytokines in the disease has led to remarkable advances in treatment, in which specific molecules have been targeted on the basis of scientific understanding. Chief among these new therapies are antagonists of TNF, which have transformed the course of the disease in many patients from one of progressive and inexorable joint destruction to one of smoldering but manageable chronic inflammation. Various other targeted therapies have been developed in the past 5 to 10 years. Blockade of cytokines other than TNF has been effective, including an antibody that blocks the IL-6 receptor, an IL-1 antagonist, and a small molecule that inhibits JAK signaling. Inhibition of T cell activation has been accomplished by blockade of B7:CD28 costimulation with CTLA-4-Ig (see Chapter 9). B cell depletion with anti-CD20 antibody has also proven to be efficacious, although the mechanisms underlying this effect are not well understood.

Multiple Sclerosis (MS)

MS is an autoimmune disease of the CNS in which $CD4^+$ T cells of the Th1 and Th17 subsets react against self myelin antigens, resulting in inflammation with activation of macrophages around nerves in the brain and spinal cord, destruction of the myelin, abnormalities in nerve conduction, and neurologic deficits. It is the most common neurologic disease of young adults. Pathologic examination reveals inflammation in the CNS white matter and demyelination. MS is characterized clinically by weakness, paralysis, and ocular symptoms with exacerbations and remissions; CNS imaging suggests that in patients with active disease, there is frequent new lesion formation.

MS is modeled by experimental autoimmune encephalomyelitis (EAE) in mice, rats, guinea pigs, and nonhuman primates, and this is one of the best characterized experimental models of an organ-specific autoimmune disease mediated mainly by T lymphocytes. EAE is induced by immunizing animals with antigens normally present in CNS myelin, such as MBP, proteolipid protein, and myelin oligodendrocyte glycoprotein, together with an adjuvant containing heat-killed mycobacteria, which is necessary to elicit a strong T cell response. About 1 to 2 weeks after immunization, animals develop encephalomyelitis, characterized by perivascular infiltrates of lymphocytes and macrophages in the CNS white matter, followed by demyelination. The neurologic lesions can be mild and self-limited or chronic and relapsing. These lesions result in progressive or remitting and relapsing paralysis. The disease also can be transferred to naive animals with T cells from diseased animals. Although antibodies against myelin antigens have been detected in patients as well as in the animal models, the pathogenic significance of these antibodies is not established.

Pathogenesis of MS

There is abundant evidence that EAE is caused by activated $CD4^+$ Th1 and Th17 cells specific for protein antigens in myelin. By analogy with the experimental disease, MS is also thought to be caused by myelin-specific Th1 and Th17 cells, and these cells have been detected in patients and isolated from the blood and CNS. How these cells are activated in patients remains an enigma. One theory is that an infection, most likely a viral infection, activates self myelin–reactive T cells by the phenomenon of molecular mimicry (see Chapter 15). Self-tolerance may fail because of the inheritance of susceptibility genes. Identical twins have a 25% to 30% concordance rate for development of MS, whereas nonidentical twins have a 6% concordance rate. These observations implicate genetic factors in the development of the disease but also indicate that genetics contributes only part of the risk. Genetic polymorphisms associated with MS include the HLA locus, with HLA-DRB1∗1501 showing the strongest linkage. Genome-wide association studies and other genomic analyses have revealed over 100 genetic variants that contribute to disease risk; most of these map to genes involved in immune function. One interesting association is with a polymorphism in the noncoding region of the gene for the IL-2 receptor α chain, CD25. This polymorphism may alter the generation and maintenance of effector T cells and/or Tregs. Other studies have suggested that the peripheral maintenance of Tregs is defective in patients with MS, but how much this contributes to a failure of self-tolerance is not known. Once myelin-specific T cells are activated, they migrate into the CNS, where they encounter myelin proteins and release cytokines that recruit and activate macrophages and more T cells, leading to myelin destruction. Studies of EAE suggest that the disease is propagated by a process known as **epitope spreading** (see Chapter 15). The tissue breakdown results in the release of new protein antigens and the expression of new, previously sequestered epitopes that activate more autoreactive T cells.

New Therapies for MS

Immunotherapy for MS has, in the past, relied on approaches whose scientific bases are not well understood. These include administration of β-interferon, which may alter cytokine responses, and treatment with a random polymer of four amino acids, which is postulated to bind to HLA molecules and block antigen presentation. Recently, however, several new immune-modifying therapies based on rational principles have been developed. One is an antibody against the the α_4 subunit of the $\alpha_4\beta_1$ integrin, also known as VLA-4 (very late antigen 4) (see Chapter 3). The antibody blocks leukocyte migration into the CNS and has been shown to be beneficial for patients. However, in a small number of patients, this treatment resulted in the reactivation of a latent JC virus infection, causing a severe and sometimes fatal CNS disease. Another recently approved drug to treat MS also interferes with leukocyte migration. The drug, called fingolimod (FTY720), blocks the sphingosine 1-phosphate–mediated pathway of T cell egress from lymphoid tissues (see Chapter 3). In a subset of patients, B cell depletion with anti-CD20 antibody is beneficial. These results suggest an important role of B cells, presumably as APCs, in the activation of pathogenic T cells. Because MBP is known to be an important self antigen that is the target of the immune response in MS, it is hoped that the administration of MBP peptides will induce antigen-specific tolerance or generate Tregs specific for the relevant antigen. It is also striking that most of the therapies are more effective in early MS, which is characterized by inflammation, than in progressive MS, which is characterized by neurodegeneration and is the major cause of permanent disability. This realization is leading to new attempts to restore myelination and repair damaged axons and neurons.

Type 1 Diabetes

Type 1 diabetes, previously called insulin-dependent diabetes, is a multisystem metabolic disease resulting from impaired insulin production that affects about 0.2% of the U.S. population, with a peak onset at 11 to 12 years of age. The incidence of the disease appears to be increasing in North America and Europe. The disease is characterized by hyperglycemia and ketoacidosis. Chronic complications of diabetes include progressive atherosclerosis of arteries, which can lead to ischemic necrosis of limbs and internal organs, and microvascular obstruction causing damage to the retina, renal glomeruli, and peripheral nerves. Type 1 diabetes is caused by a deficiency of insulin resulting from immune-mediated destruction of the insulin-producing β cells of the islets of Langerhans in the pancreas, and continuous hormone replacement therapy is needed. There is usually a long lag of many years between the initiation of autoimmunity and overt clinical disease because 90% or more of the islets have to be destroyed before clinical manifestations are seen.

Pathogenesis of Type 1 Diabetes

Several mechanisms may contribute to β cell destruction, including inflammation mediated by $CD4^+$ Th1 cells reactive with islet antigens (including insulin), CTL-mediated lysis of islet cells, local production of cytokines (TNF and IL-1) that damage islet cells, and autoantibodies against islet cells. In the few cases in which the pancreatic lesions have been examined at the early active stages of the disease, the islets show cellular necrosis and lymphocytic infiltration consisting of both $CD4^+$ and $CD8^+$ T cells. This lesion is called insulitis. Autoantibodies against islet cells and insulin are also detected in the blood of these patients. In susceptible children who have not developed diabetes (such as relatives of patients), the presence of antibodies against islet cells is predictive of the development of type 1 diabetes. An informative animal model of the disease is the nonobese diabetic (NOD) mouse, which develops spontaneous diabetes. In this model, there is evidence for defective survival and function of Tregs and resistance of effector T cells to suppression by Tregs. Another interesting idea that has emerged mostly from the mouse model is that post-translational modification of islet antigens may lead to the creation of new epitopes that elicit immune responses, similar to the neoantigens in RA, discussed previously.

Multiple genes are associated with type 1 diabetes. Most attention has been focused on the role of HLA genes. Between 90% and 95% of Caucasians with type 1 diabetes have HLA-DR3 or DR4, or both, in contrast to about 40% of healthy subjects, and 40% to 50% of patients are DR3/DR4 heterozygotes, in contrast to 5% of healthy subjects. The actual HLA genes that may play a role in the pathogenesis may be HLA-DQ alleles that are in linkage disequilibrium with the DR alleles. Several non-HLA genes also contribute to the disease. The first of these to be identified is insulin, with tandem repeats in the promoter region being associated with disease susceptibility. The mechanism of this association is unknown; it may be related to the level of expression of insulin in the thymus, which determines whether insulin-specific T cells will be deleted (negatively selected) during their maturation. Several other polymorphisms have been identified in patients and in NOD mice, including in the *IL2* and *CD25* genes. Different polymorphisms in these genes may increase or decrease the risk for developing the disease, but how these polymorphisms affect T cell responses is not fully established. Some studies have suggested that viral infections (e.g., with coxsackievirus B4) may precede the onset of type 1 diabetes, perhaps by initiating cell injury, inducing inflammation and the expression of costimulators, and triggering an autoimmune response. However, epidemiologic data suggest that repeated infections protect against type 1 diabetes, and this is similar in the NOD model. In fact, it has been postulated that one reason for the increasing incidence of type 1 diabetes in higher-income countries is the control of infectious diseases.

New Therapies for Type 1 Diabetes

The most interesting new therapeutic strategies for type 1 diabetes are focused on inducing tolerance with diabetogenic peptides from islet antigens (such as insulin) and inducing or giving Tregs to patients. These clinical trials are in their early stages.

Inflammatory Bowel Disease

IBD is a heterogeneous group of disorders characterized by chronic remitting inflammation in the small or large bowel that is likely a result of inadequately regulated responses to commensal bacteria. The two main types of IBD are **Crohn's disease,** which can affect the entire thickness of the wall in any part of the gastrointestinal tract but most frequently involves the terminal ileum, and **ulcerative colitis,** which is restricted to the colonic mucosa.

Pathogenesis of IBD

Although the causes of Crohn's disease and ulcerative colitis are poorly understood, several types of evidence suggest that these disorders are a result of defects in the regulation of immune responses to commensal organisms in the gut in genetically susceptible individuals. A number of immunologic abnormalities may contribute to the development of IBD (Fig. 19.12).

- Defects in innate immunity to gut commensals. Loss-of-function mutations in the gene that encodes the NOD2 cytoplasmic innate immune sensor are associated with a subset of Crohn's disease and may lead to reduced innate defenses against intestinal microbes (see Chapters 4 and 14). There also may be defective expression of molecules such as defensins, leading to increased commensal bacterial invasion through the intestinal epithelium.
- Abnormal Th17 and Th1 responses. Analysis of T cell responses in animal models and patients with IBD indicates that there is an active Th17 response in the affected parts of the bowel. Genetic studies have shown that polymorphisms in genes encoding the IL-23 receptor carry increased risk for IBD, although the effect of the polymorphisms on expression or function of the receptor are not known. Crohn's disease is also characterized by granulomatous inflammation driven by interferon (IFN)-γ–producing Th1 cells.
- Defective function of regulatory T cells. It is possible that IBD may be caused by inadequate Treg-mediated suppression of immune responses to commensal organisms. The evidence supporting this hypothesis originally came from mouse models in which an absence of Tregs leads to colitis. In humans, *FOXP3* mutations result in a failure to develop Tregs and cause the disease called

IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, which includes severe gut inflammation in addition to autoimmune involvement of many other tissues. Mutations in the receptor for IL-10, an immune-suppressive cytokine made by Tregs (and other cells) cause early-onset severe colitis.

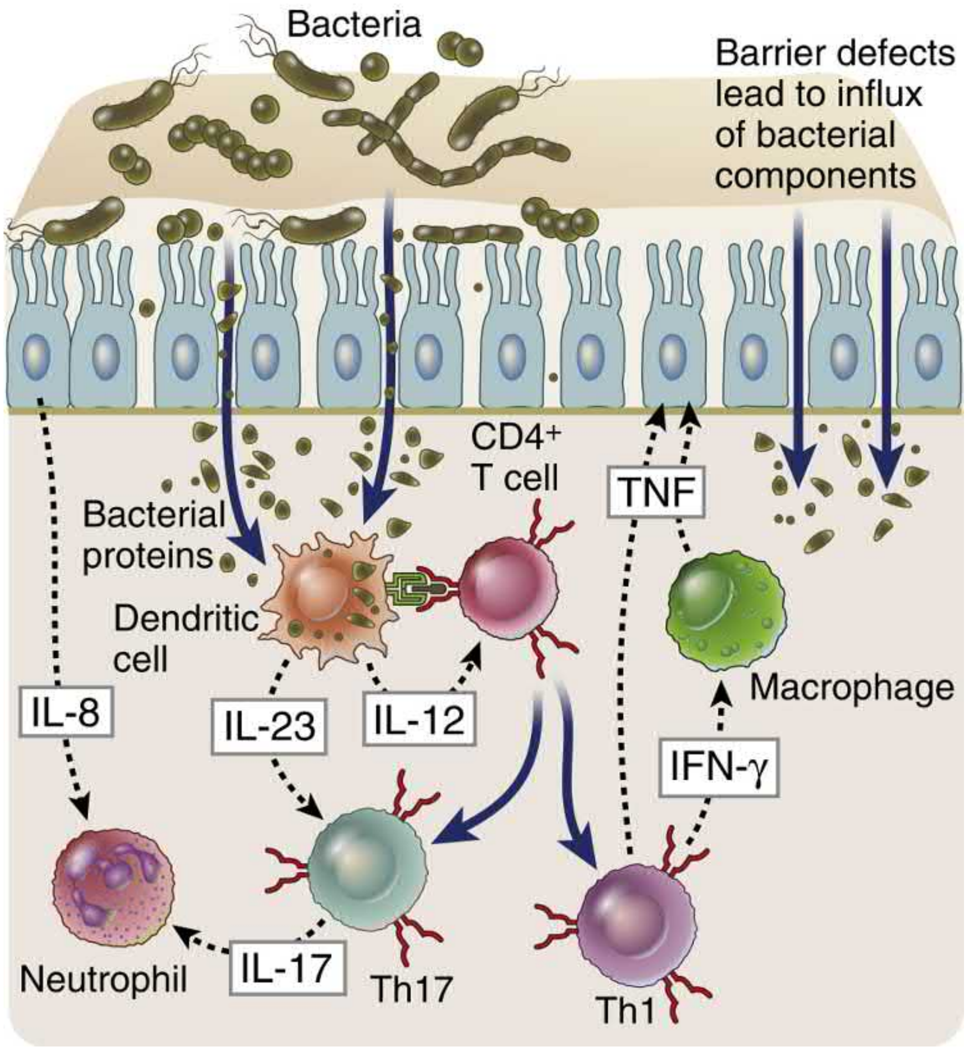
Bacteria
Barrier defects lead to influx of bacterial components
CD4+ T cell
TNF
Bacterial proteins
Dendritic cell
Macrophage
IL-8
IL-23
IL-12
IFN-γ
Neutrophil
IL-17
Th17
Th1

FIGURE 19.12 Postulated pathogenesis of Crohn's disease.Bacteria from the intestinal lumen enter into the lamina propria, where they induce the development of Th1 and Th17 cells. Cytokines produced by these cells cause inflammation and tissue damage. *IFN-γ,* Interferon-γ; *TNF,* tumor necrosis factor.

- Polymorphisms of genes that are associated with macroautophagy and the unfolded protein response to endoplasmic reticulum stress are risk factors for IBD. Macroautophagy is a process in which cells sequester cytoplasmic organelles within autophagosomes, which then fuse with lysosomes, promoting the destruction of the organelles. Variants of autophagy genes (including *ATG16L1* and *IRGM*) that are associated with Crohn's disease impair autophagy in Paneth cells, and for unclear reasons, this reduces secretion of lysozyme and defensins into the intestinal lumen.

Immunotherapies for IBD

TNF antagonists were the first biologic agent used to treat IBD. The findings of exaggerated Th1 and Th17 responses are the basis for treating patients with a monoclonal antibody that binds a polypeptide (p40) shared by IL-23 and IL-12. IL-23 is required for Th17-mediated immune responses, and IL-12 is required for Th1 responses (see Chapter 10). Clinical trials of IL-17 antagonist treatment for Crohn's disease have not shown efficacy, suggesting that excessive production of IL-17 may not, by itself, be responsible for this disorder. Another biologic agent approved for Crohn's disease is a monoclonal antibody specific for the $\alpha_4\beta_7$ integrin, which is expressed on gut-homing lymphocytes.

Celiac Disease

Celiac disease (gluten-sensitive enteropathy) is an inflammatory disease of the small bowel mucosa caused by immune responses against ingested gliadin, a major protein component of the broader group of proteins called gluten present in wheat and other grains. Celiac disease is characterized by chronic inflammation in the small bowel mucosa, leading to atrophy of villi, malabsorption, and various nutritional deficiencies that lead to extraintestinal manifestations. The disease is treated by restricting diets to gluten-free foods.

Pathogenesis of Celiac Disease

$CD4^+$ T cell responses to gliadin are likely involved in disease pathogenesis (Fig. 19.13). T cells specific for gliadin peptides are found in patients with celiac disease, and the inflammatory process in the bowel includes T cells and T cell cytokines. The risk of developing celiac disease is strongly associated with HLA-DQ2 and DQ8 alleles, and there is evidence that these class II HLA molecules can present modified gluten peptides to mucosal CD4$^+$ T cells in affected individuals. A host enzyme transglutaminase 2 (TG2) converts a neutral amino acid glutamine in gluten peptides to the negatively charged residue glutamic acid; the negatively charged peptides bind more efficiently to DQ2 and DQ8 and activate specific T cells that secrete cytokines that contribute to intestinal inflammation. Patients produce IgA and IgG antibodies specific for gluten as well as autoantibodies specific for TG2. Whether these antibodies contribute to disease development is not known, but they are a diagnostic marker for the disease. In addition to CD4$^+$ T cell responses, killing of intestinal epithelial cells by CD8$^+$ CTLs and natural killer (NK) cells may also contribute to the disease, although the source of the peptides recognized by the CTLs or what the NK cells are responding to is not clear.

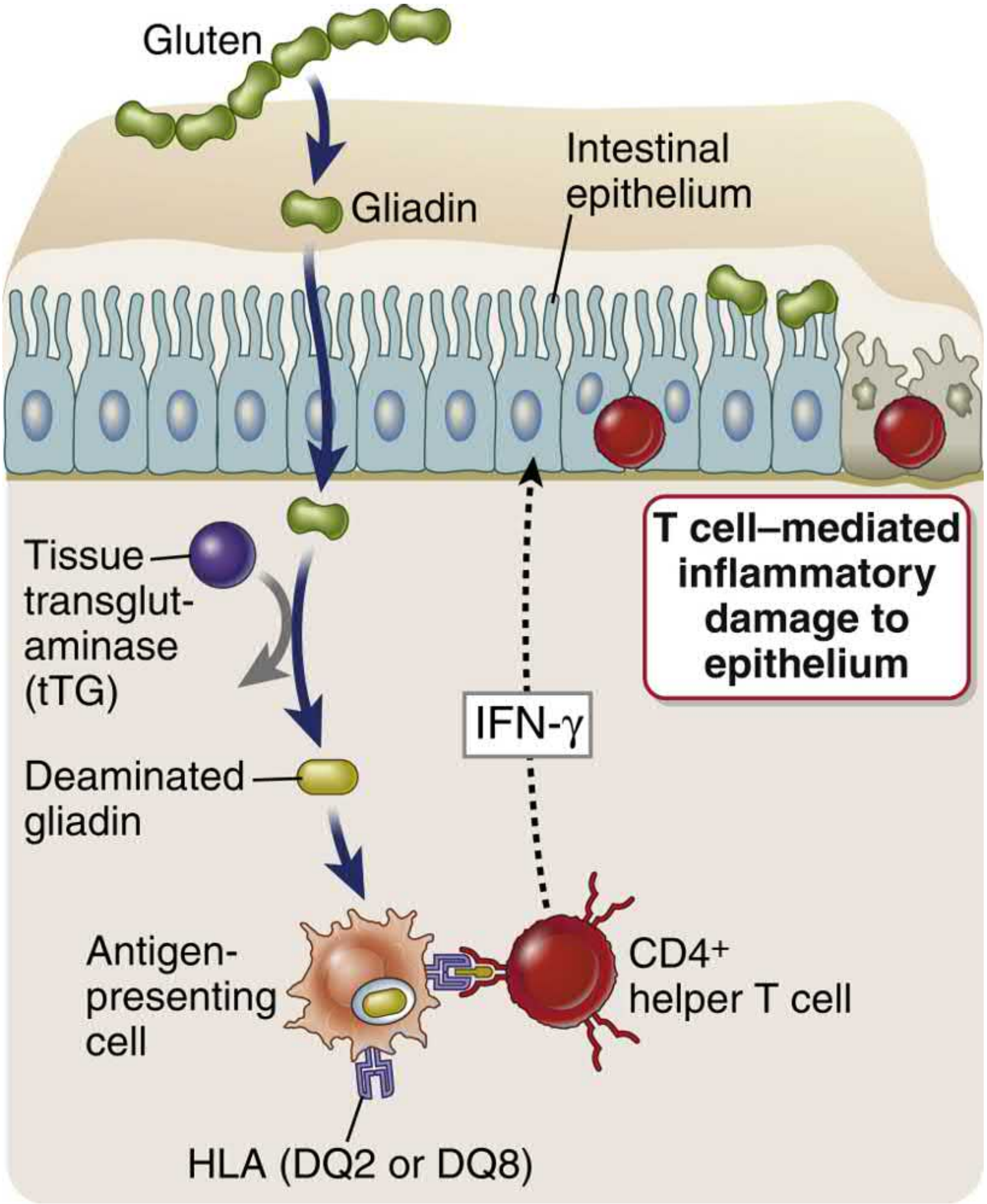
Gluten
Gliadin
Intestinal epithelium
Tissue transglutaminase (tTG)
T cell–mediated inflammatory damage to epithelium
IFN-γ
Deaminated gliadin
Antigen-presenting cell
CD4+ helper T cell
HLA (DQ2 or DQ8)

FIGURE 19.13 Postulated pathogenesis of celiac disease. Gliadin is converted to a peptide that is presented by lamina propria dendritic cells to $CD4^+$ T lymphocytes. Cytokines produced by the T cells damage the intestinal epithelium. *HLA,* Human leukocyte antigen; *IFN-γ,* interferon-γ.

Psoriasis

Psoriasis is the prototypic IL-17–mediated chronic inflammatory autoimmune disease. It involves primarily the skin and also affects the joints and other tissues in some cases. The responsible self antigens are not clearly defined, but possible candidates include cathelicidin (an antimicrobial protein) and a keratin, both produced by keratinocytes, and other proteins made by melanocytes. The autoimmune response may be triggered by infection or other unknown stimuli. Several lines of evidence have established the central role of IL-17–producing cells in the resulting inflammation. High levels of IL-17 and the Th17-inducing cytokine IL-23 are found in psoriatic lesions, as are large numbers of IL-17–producing $CD4^+$ and $CD8^+$ T cells. The contribution of IL-17–producing $\gamma\delta$ T cells and ILCs has been suggested but not clearly established. Genome-wide association studies have revealed disease-associated polymorphisms in the IL-23 receptor gene and other genes associated with Th17 development. It is postulated that once IL-17–producing T cells are activated, presumably by one or more self antigens, the IL-17 they produce stimulates inflammation and activates DCs to produce TNF and other, Th17-inducing cytokines. This reaction sets up a vicious cycle of continuing inflammation. Effective new biologic therapies have been developed based on this model. The first such agents to be used in the disease were TNF antagonists. These were followed by an antibody specific for the p40 chain that is shared by IL-12 and IL-23, mentioned earlier in the therapy of IBD. The most successful of these biologic agents are antibodies that block IL-17 or IL-23, which are very effective in most patients.

Summary

- Disorders caused by abnormal immune responses are called hypersensitivity diseases. Pathologic immune responses may be autoimmune responses directed against self antigens or uncontrolled and excessive responses to foreign (e.g., microbial) antigens.

- Hypersensitivity diseases may result from antibodies that bind to cells or tissues (type II hypersensitivity), circulating immune complexes that are deposited in tissues (type III), or T lymphocytes reactive with antigens in tissues (type IV). Immediate hypersensitivity (type I) reactions are the cause of allergic diseases and are described in Chapter 20.
- The effector mechanisms of antibody-mediated tissue injury are complement activation and Fc receptor–mediated inflammation. Some antibodies cause disease by opsonizing host cells for phagocytosis or by interfering with normal cellular functions without producing tissue injury.
- The effector mechanisms of T cell–mediated tissue injury are inflammatory reactions induced by cytokines secreted mainly by $CD4^+$ Th1 and Th17 cells and cell lysis by cytotoxic T lymphocytes. The classical T cell–mediated reaction is delayed-type hypersensitivity, induced by activation of previously primed T cells and the production of cytokines that recruit and activate various leukocytes, predominantly macrophages.
- The current treatment of autoimmune diseases is targeted at reducing immune activation and the injurious consequences of the autoimmune reaction. Agents include those that block inflammation, such as antibodies against cytokines and integrins, and those that block lymphocyte activation or destroy lymphocytes. A future goal of therapy is to inhibit the responses of lymphocytes specific for self antigens and to induce tolerance in these cells.
- Autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, type 1 diabetes, inflammatory bowel disease, celiac disease, and psoriasis illustrate many of the effector mechanisms that cause tissue injury in hypersensitivity reactions and the

roles of susceptibility genes and environmental factors in the development of these disorders.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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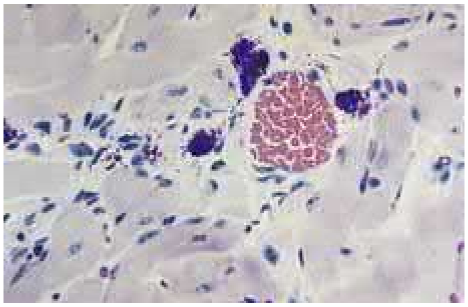


Chapter 20: Allergy

Overview of IgE-Dependent Allergic Reactions,

Production of IgE,
- The Nature of Allergens,
- Activation of Type 2 Cytokine–Producing Helper T Cells,
- Activation of B Cells and Switching to IgE,

Cells Involved in Allergic Reactions,
- Role of Th2 Cells and Innate Lymphoid Cells in Allergic Disease,
- Properties of Mast Cells and Basophils,
- Properties of Eosinophils,

Reactions Dependent on IgE and Mast Cells,
- The Immediate Reaction,
- The Late-Phase Reaction,

Genetic Susceptibility to Allergic Disease,
- Environmental Factors in Allergy,

Allergic Diseases in Humans: Pathogenesis and Therapy,
- Systemic Anaphylaxis,

Asthma,
Immediate Hypersensitivity Reactions in the Upper Respiratory Tract, Gastrointestinal Tract, and Skin,
Specific Immunotherapy (Desensitization) for Allergic Diseases,

The Protective Roles of Immune Reactions Mediated by IgE and Mast Cells,

Summary,

A variety of human diseases are caused by immune responses to nonmicrobial environmental antigens and involve the type 2 cytokines interleukin-4 (IL-4), IL-5, and IL-13 produced by Th2 cells and group 2 innate lymphoid cells (ILC2s), IgE (immunoglobulin E), mast cells, and eosinophils. In the effector phase of these responses, mast cells and eosinophils are activated to rapidly release mediators that cause increased vascular permeability, vasodilation, and bronchial and visceral smooth muscle contraction. This vascular reaction is called **immediate (type I) hypersensitivity** because it begins rapidly, within minutes of antigen challenge, in a previously sensitized individual (immediate) and has pathologic consequences (hypersensitivity). After the immediate response, there is a more slowly developing inflammatory component called the **late-phase reaction** characterized by the accumulation of mainly eosinophils and neutrophils. In clinical medicine, these reactions are called **allergy** or **atopy,** and the associated diseases are called allergic, atopic, or immediate hypersensitivity diseases. (The term allergy is often imprecisely used in clinical practice to describe other hypersensitivity reactions to environmental antigens, such as contact hypersensitivity, which is a T cell– and cytokine-mediated inflammatory reaction.) Repeated bouts of IgE- and mast cell–dependent reactions can lead to chronic allergic diseases, with tissue damage and remodeling. The most common of these chronic disorders are atopic dermatitis, hay fever (allergic rhinitis), and asthma. The antigens that elicit allergic reactions are called **allergens.** Most of them are common environmental proteins produced by animals or plants, and chemicals, including drugs, that can modify self proteins.

Although the term atopy was coined to denote a reaction that is "out of place" (unusual), we now realize that allergy is the most common disorder of immunity, affecting about 30% of all individuals in the United States and Europe, and its prevalence is increasing worldwide. In this chapter, we will describe the sequence of events that lead to production of type 2 cytokines and IgE, mast cell activation, and the roles of various mediators in immediate hypersensitivity. We will then describe selected clinical syndromes associated with allergic reactions and the principles of therapy for these diseases. We conclude with a discussion of the physiologic role of IgE-mediated immune reactions in host defense.

Overview of IgE-Dependent Allergic Reactions

All allergic reactions share some common features, although they differ greatly in the types of antigens that elicit the reactions and their clinical and pathologic manifestations.

Allergy is the prototypic type 2 inflammatory disease, mediated by the cytokines IL-4, IL-5, and IL-13, different combinations of which are secreted by Th2 cells, T follicular helper (Tfh) cells, ILC2s, and a few other cell types. The cytokine responses of these cells are often collectively called **type 2 immune responses.** Many of the early events and pathologic features of the reaction are triggered by these cytokines, which may be produced by Tfh cells in lymphoid organs and by ILC2s and Th2 cells in tissues. Delayed-type hypersensitivity (DTH), described in Chapter 19, is the classical type 1 inflammatory reaction and differs in many respects from allergy.

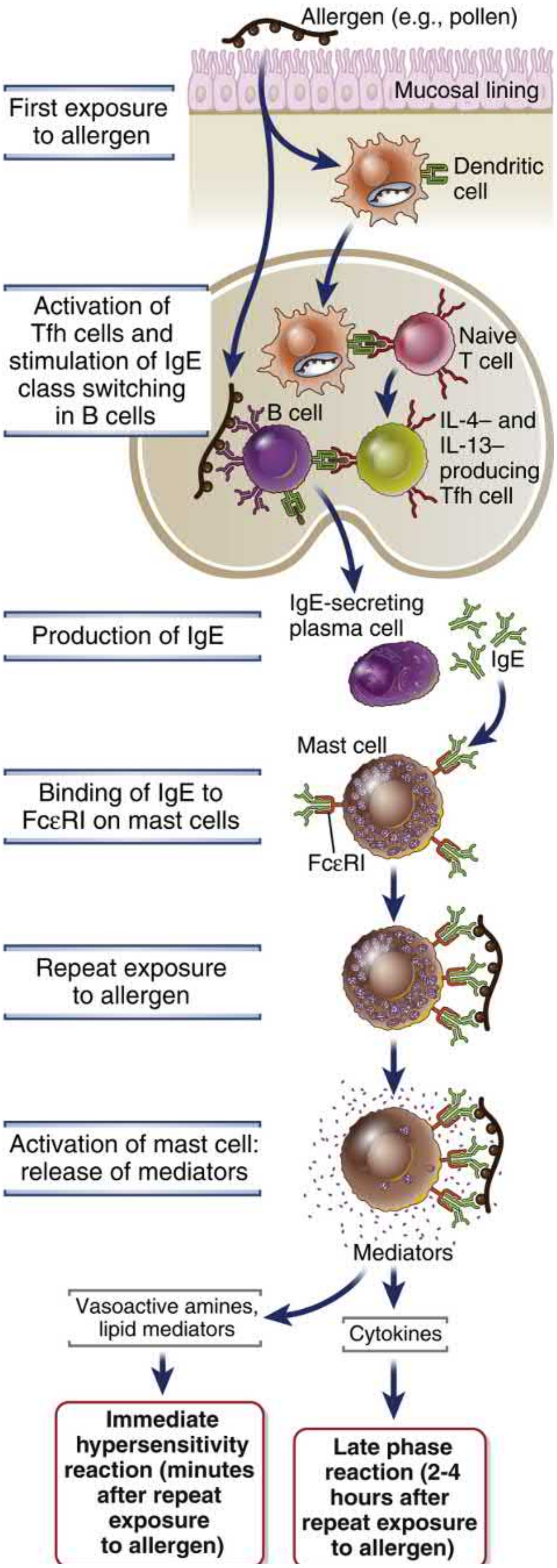
A hallmark of allergic diseases is the production of IgE antibody, which depends on the activation of IL-4– and IL-13–producing helper T cells. Whereas healthy individuals either do not respond to, or have only harmless T cell and antibody responses to, common environmental antigens, atopic individuals develop strong type 2 helper T cell responses and produce IgE on exposure to these substances.

Allergic reactions require previous T cell–dependent allergen-specific IgE production by B cells and the binding of the IgE to mast cells. The typical sequence of events leading to an immediate hypersensitivity reaction is illustrated in Fig. 20.1. Helper T cell–dependent IgE produced in response to the allergen binds to Fc receptors on mast cells; this process is called **sensitization** of mast cells. Re-exposure to the allergen then activates the mast cells to release mediators that cause the harmful reaction. We will describe each of these steps in detail later in the chapter.

The clinical and pathologic manifestations of allergy consist of the vascular and smooth muscle reactions that develop rapidly after allergen challenge in a sensitized individual (immediate hypersensitivity) and a delayed late-phase inflammatory reaction. All of these reactions may be initiated by IgE-mediated mast cell activation, but different mediators are responsible for the immediate and late-phase reactions. Because mast cells are abundant in connective tissues and under epithelial barriers, these tissues are the most common sites of allergic reactions. Some immediate hypersensitivity reactions may be triggered by nonimmunologic stimuli, such as exercise, cold temperatures, and several drugs. These stimuli induce mast cell degranulation and the release of mediators without antigen exposure or IgE production. Such reactions are said to be nonatopic.

Allergic reactions are manifested in different ways, depending on the tissues affected, including skin rashes, sinus and nasal congestion, inflamed conjunctiva, bronchial constriction with difficulty in breathing, abdominal pain, diarrhea, and shock. In the most extreme systemic form, called **anaphylaxis,** mast cell–derived mediators can restrict airways to the point of asphyxiation and produce cardiovascular collapse leading to shock, which together may result in death. (The term anaphylaxis was coined to indicate that antibodies, in these cases IgE antibodies, could confer the opposite of protection [prophylaxis] on an unfortunate individual.) We will return to the pathogenesis of these reactions later in the chapter.

The development of allergies is the result of complex and poorly understood gene-environment interactions. There is a genetic predisposition for the development of allergies, and relatives of allergic individuals are more likely to have allergies than unrelated people, even when they do not share environments. Many susceptibility genes have been identified that we will discuss later in this chapter. Various environmental factors besides the exposure to allergens, especially in industrialized societies, including air pollution and exposure to microbes, have a profound influence on the propensity to develop allergies.



Allergen (e.g., pollen)
Mucosal lining
First exposure to allergen
Dendritic cell
Activation of Tfh cells and stimulation of IgE class switching in B cells
Naive T cell
B cell
IL-4– and IL-13–producing Tfh cell
Production of IgE
IgE-secreting plasma cell
IgE
Mast cell
Binding of IgE to FcεRI on mast cells
FcεRI
Repeat exposure to allergen
Activation of mast cell: release of mediators
Mediators
Vasoactive amines, lipid mediators
Cytokines
Immediate hypersensitivity reaction (minutes after repeat exposure to allergen)
Late phase reaction (2-4 hours after repeat exposure to allergen)

FIGURE 20.1 Sequence of events in immediate hypersensitivity reactions.Immediate hypersensitivity diseases are initiated by the introduction of an allergen, which stimulates IL-4– and IL-13–producing helper T cell responses and IgE production. IgE sensitizes mast cells by binding to FcεRI, and subsequent exposure to the allergen activates the mast cells to secrete the mediators that are responsible for the pathologic reactions of immediate hypersensitivity. *Tfh,* T follicular helper cells.

With this introduction, we will proceed to a description of the steps leading to reactions of immediate hypersensitivity.

Production of IgE

Atopic individuals produce high levels of IgE in response to environmental allergens, whereas normal individuals generally produce other Ig isotypes, such as IgM and IgG, and only small amounts of IgE. IgE is of central importance in allergy because this isotype is responsible for sensitizing mast cells. IgE is the antibody isotype that contains the ε heavy chain (see Chapter 5). It binds to specific Fc receptors on mast cells and activates these cells upon antigen binding. The quantity of IgE synthesized depends on the propensity of an individual to generate allergen-specific Tfh cells that produce IL-4 and IL-13, because these cytokines stimulate B cell antibody class switching to IgE (see Chapter 12). The development of IL-4– and IL-13–expressing T cell responses against particular antigens may be influenced by a variety of factors, including the nature of the antigens, the history of antigen exposure, and inherited genes.

The Nature of Allergens

Antigens that elicit immediate hypersensitivity reactions (allergens) are proteins or chemicals bound to proteins. Typical allergens include proteins in pollen, house dust mites, animal dander, foods, and drugs. It is not known why some antigens induce IL-4–producing helper T cell responses and allergic reactions whereas others do not. Two important characteristics of allergens are that individuals are exposed to them repeatedly and, unlike microbes, they do not generally stimulate the types of innate immune responses that are associated with macrophage and dendritic cell (DC) secretion of Th1- and Th17-inducing cytokines.

The ability of an antigen to trigger allergic reactions also may be related to its chemical nature. Although no structural characteristics of proteins can definitively predict whether they will be allergenic, some features are typical of many common allergens. These include low-to-medium molecular weight (5 to 70 kD), stability, glycosylation, and solubility in body fluids. These structural features probably protect the antigens from denaturation and allow them to be absorbed intact and disseminate widely. Curiously, many allergens, such as the cysteine protease of the house dust mite and phospholipase A2 (PLA_2) in bee venom, are enzymes, but the importance of the enzymatic activity to their role as allergens is not known.

Because immediate hypersensitivity reactions are dependent on $CD4^+$ T cells, T cell–independent antigens, such as polysaccharides, cannot elicit these reactions unless they become attached to proteins. Some nonprotein substances, such as penicillin, can elicit strong IgE responses. These molecules react chemically with amino acid residues in self proteins to form hapten-carrier conjugates (see Chapter 12), which induce type 2 helper T cell responses and IgE production.

The natural history of antigen exposure is an important determinant of the amount of specific IgE antibodies produced and the subsequent activation of mast cells. Repeat exposure to a particular antigen is necessary for development of an allergic reaction to that antigen because switching to the IgE isotype and sensitization of mast cells with IgE must happen before an immediate hypersensitivity reaction to an antigen can occur. Individuals with allergic rhinitis or asthma often benefit from a geographic change of residence with a change in indigenous plant pollens, although environmental antigens in the new residence may trigger an eventual return of the symptoms. A dramatic example of the importance of repeated exposure to antigen in allergic disease is seen in cases of bee sting allergy. The proteins in the insect venoms are not usually of concern on the first encounter because an atopic individual has no preexisting specific IgE antibodies. However, IgE may be produced after a single encounter with antigen with no harmful consequences, and a second sting by an insect of the same species may induce fatal anaphylaxis! Similarly, exposures to small amounts of peanuts can trigger fatal reactions in previously sensitized individuals.

Activation of Type 2 Cytokine–Producing Helper T Cells

The development of allergic disease begins with the differentiation of IL-4–, IL-5–, and IL-13–producing CD4$^+$ helper T cells in lymphoid tissues. The signals that drive the differentiation of naive CD4$^+$ T cells into Th2 cells and IL-4– and IL-13–producing Tfh cells in response to most environmental antigens are not established. As discussed later, there is a strong genetic propensity to make type 2 responses against some allergens, but this alone does not explain why atopic individuals are prone to developing such responses. In some chronic allergic diseases, an initiating event may be epithelial barrier injury, which results in local production of Th2-inducing cytokines. For instance, in a chronic allergic reaction of the skin called atopic dermatitis, the epithelial barrier abnormality is usually not visible and of unknown cause, but sometimes it is related to inherited deficiency of filaggrin, which is a keratinocyte protein needed to maintain normal barrier function of the skin. In the bronchial tree of the lung, viral infections are considered a major cause of the initial injury. In both tissues, injury induces epithelial cells to secrete IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). DCs exposed to these cytokines are mobilized to migrate to lymph nodes and to drive differentiation of naive T cells in the lymph nodes toward IL-4–, IL-5–, and IL-13–producing Th2 and Tfh cells. IL-25, IL-33, and TSLP also activate ILC2s to upregulate GATA3, which enhances the transcription and secretion of IL-5 and IL-13. Thus, epithelial cell–derived cytokines may provide a link between allergens and type 2 responses.

The differentiated Th2 cells migrate to tissue sites of allergen exposure, where they contribute to the inflammatory phase of allergic reactions, described later. Tfh cells remain in lymphoid organs, where they help B cells.

Activation of B Cells and Switching to IgE

B cells specific for allergens are activated by Tfh cells in secondary lymphoid organs, as in other T cell–dependent B cell responses (see Chapter 12). In response to CD40 ligand and cytokines, mainly IL-4 and IL-13, produced by these helper T cells, the B cells undergo heavy chain isotype switching and produce IgE. IgE circulates as a bivalent antibody and is normally present in plasma at a concentration of less than 150 ng/mL. In pathologic conditions such as helminthic infections and severe atopy, this level can increase significantly. Allergen-specific IgE produced by plasmablasts and plasma cells enters the circulation and binds to Fc receptors on tissue mast cells and circulating basophils, so that these cells are sensitized and poised to react to a subsequent encounter with the allergen.

Cells Involved in Allergic Reactions

The major effector cells of immediate hypersensitivity reactions and allergic disease are type 2 cytokine-secreting cells (Th2 cells, Tfh cells, and possibly ILC2s), mast cells, basophils, and eosinophils. Although each of these cell types has unique characteristics, all four secrete mediators involved in allergic reactions. Tfh cells stimulate IgE production in secondary lymphoid organs, and Th2 cells and ILC2s contribute to tissue inflammation by secreting cytokines. Mast cells, basophils, and eosinophils release their granule contents and produce other mediators, all of which are responsible for the pathologic consequences of allergic reactions. In this section, we will discuss the roles of these cell types in allergy.

Role of Th2 Cells and Innate Lymphoid Cells in Allergic Disease

Th2 cells and ILC2s secrete cytokines, including IL-4, IL-5, and IL-13, which promote inflammatory responses to allergens within tissues. The general properties of Th2 cells and the signals that drive their differentiation from naive T cells were discussed in Chapter 10. IL-4 secreted by Th2 cells induces expression of endothelial VCAM-1 (vascular cell adhesion molecule 1), which promotes the recruitment of eosinophils and additional Th2 cells into tissues. IL-5 secreted by Th2 cells enhances eosinophil production in the bone marrow and activates mature eosinophils in tissues. IL-13 stimulates epithelial cells (e.g., in the airways) to secrete increased amounts of mucus, and excessive mucus production is also a common feature of these reactions.

Consistent with a central role of Th2 cells in immediate hypersensitivity, more allergen-specific IL-4–secreting T cells are found in the blood of atopic individuals than in nonatopic persons. In atopic patients, the allergen-specific T cells also produce more IL-4 per cell than in normal individuals. In animal models, a disease resembling human asthma can be induced by generation of Th2 cells specific for an inhaled antigen or by adoptive transfer of these cells into naive mice. Accumulations of Th2 cells are found at sites of immediate hypersensitivity reactions in the skin and bronchial mucosa.

ILC2s produce many of the same cytokines as Th2 cells, specifically IL-5 and IL-13, and therefore may have similar roles in allergic reactions. Because ILCs normally reside in tissues, their cytokines may contribute to early allergic inflammation before Th2 cells are generated and migrate to the tissues. The ILC2s may also work in concert with Th2 cells later, to sustain inflammation.

Properties of Mast Cells and Basophils

Mast cells, basophils, and eosinophils are myeloid cells that share some features but differ phenotypically and functionally in significant ways (Table 20.1). All mast cells are derived from progenitors in the bone marrow. Normally, mature mast cells are not found in the circulation. Progenitors migrate to the peripheral tissues as immature cells and undergo differentiation in response to local biochemical cues, including stem cell factor released by tissue cells, which binds to the c-Kit receptor on the mast cell precursors. Mature mast cells are found throughout the body, predominantly near blood vessels (Fig. 20.2A) and nerves and beneath epithelia. They are also present in lymphoid organs. Human mast cells vary in shape and have round nuclei, and the cytoplasm contains membrane-bound granules and lipid bodies. The granules contain acidic proteoglycans that bind basic dyes.

Activated mast cells secrete a variety of mediators that are responsible for the manifestations of allergic reactions (Table 20.2) **.** These include substances that are stored in granules and rapidly released upon activation and others that are synthesized upon activation and secreted. The production and actions of these mediators are described later.

Subsets of mast cells have been described in mice and humans, which differ in their main location (mucosa versus connective tissue in mice), or granule protease content (tryptase only or tryptase and chymase in humans). However, heterogeneity of mast cells appears to be greater than just two subsets and does not reflect distinct developmental lineages, but rather results from different gene expression profiles induced by microenvironmental factors and stimuli that vary between tissues.

Basophils are blood granulocytes with structural and functional similarities to mast cells. Like other granulocytes, basophils are derived from bone marrow progenitors (which are different from the precursors of mast cells), mature in the bone marrow, and circulate in the blood (Fig. 20.2B). Basophils constitute 0.5% or less of blood leukocytes. Although they are normally not present in tissues, basophils may be recruited to some inflammatory sites. Basophils contain granules that bind basic dyes, and they are capable of synthesizing many of the same mediators as mast cells (see Table 20.2). Like mast cells, basophils express Fcε receptor type I (FcεRI), bind IgE, and can be triggered by antigen binding to the IgE. Therefore, basophils that are recruited into tissue sites where antigen is present may contribute to immediate hypersensitivity reactions.

Table 20.1

Characteristic	Mast Cells	Basophils	Eosinophils
Major site of maturation	Bone marrow precursors mature in connective tissue and mucosal tissues	Bone marrow	Bone marrow
Location of cells	Connective tissue and mucosal tissues	Blood (~0.5% of blood leukocytes); recruited into tissues	Blood (~2% of blood leukocytes); recruited into tissues
Life span	Weeks to months	Days	Days to weeks
Major growth and differentiation factor (cytokines)	Stem cell factor, IL-3	IL-3	IL-5
Expression of FcεRI	High	High	Low
Major granule contents	Histamine, heparin and/or chondroitin sulfate, proteases	Histamine, chondroitin sulfate, protease	Major basic protein, eosinophil cationic protein, peroxidases, hydrolases, lysophospholipase

FcεRI, Fcε receptor type I; *IL*, interleukin.

Binding of IgE to Mast Cells and Basophils: the Fcε Receptor

Mast cells and basophils express a high-affinity Fc receptor specific for ε heavy chains, called Fc ε RI, which binds IgE. IgE, like all other antibodies, is made exclusively by B cells, yet IgE functions as an antigen receptor on the surface of mast cells and basophils. This function is accomplished by IgE binding to FcεRI on these cells. The affinity of FcεRI for IgE is very high (dissociation constant [K_d] of approximately 1×10^{-10} M), higher than that of any other Fc receptor for its antibody ligand. Therefore, although the normal serum concentration of IgE is low compared to other Ig isotypes ($<5 \times 10^{-10}$ M), there is full occupancy of FcεRI receptors by IgE, and the majority of mast cells are always coated with IgE, even in nonatopic individuals.

Each FcεRI molecule on mast cells is composed of an α chain that binds the Fc region of IgE and a β chain and two γ chains that are responsible for signaling (Fig. 20.3) **.** The amino-terminal extracellular portion of the α chain includes two Ig-like domains that form the binding site for IgE. The β chain of FcεRI contains a single immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasmic carboxy-terminal domain. The two identical γ chain polypeptides are linked by a disulfide bond and are homologous to the ζ chain of the T cell antigen receptor complex (see Chapter 7). The cytoplasmic portion of each γ chain contains one ITAM. The same γ chain serves as the signaling subunit for FcγRI, FcγRIIIA, and FcαR and is called the FcR γ chain (see Chapter 13). Tyrosine phosphorylation of the ITAMs of the β and γ chains initiates the signaling cascade from the receptor that is required for mast cell activation, described shortly.

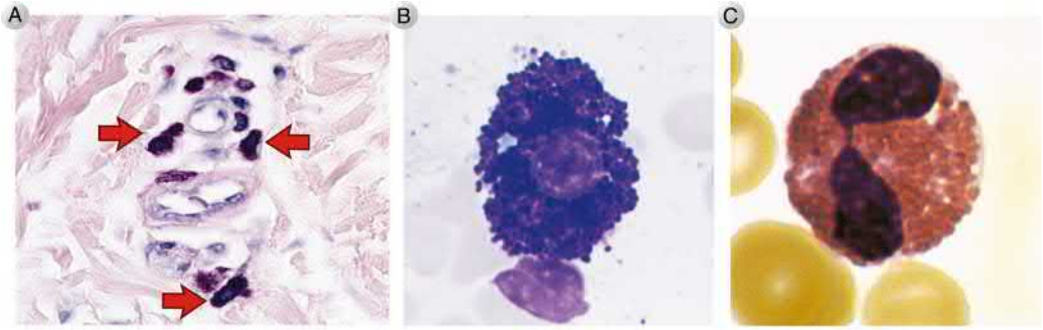


FIGURE 20.2 Morphology of mast cells, basophils, and eosinophils. Photomicrographs of Wright-Giemsa–stained perivascular dermal mast cells (**A,** *arrows*), peripheral blood basophil **(B),** and peripheral blood eosinophil **(C)** are presented. Note the characteristic *blue-staining* cytoplasmic granules of the basophil and *red staining* of the cytoplasmic granules in the eosinophil.

A, Courtesy Dr. George Murphy. *B* and *C,* Courtesy Dr. Jonathan Hecht, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.

Table 20.2

Cell Type	Mediator Category	Mediator	Function/Pathologic Effects
Mast cells and basophils	Stored preformed in cytoplasmic granules	Histamine	Increase vascular permeability; stimulate smooth muscle cell contraction
		Enzymes: neutral proteases (tryptase and/or chymase), acid hydrolases, cathepsin G, carboxypeptidase	Degradation of microbial structures; tissue damage/remodeling
	Major lipid mediators produced on activation	PGD_2	Vasodilation; bronchoconstriction; leukocyte chemotaxis
		Leukotrienes C_4, D_4, E_4	Prolonged bronchoconstriction; mucus secretion; increased vascular permeability
		PAF	Vasodilation; increased vascular permeability; leukocyte adhesion, chemotaxis, degranulation, oxidative burst
	Cytokines produced on activation	IL-3, TNF, MIP-1α	Mast cell proliferation; inflammation (late-phase reaction)
		IL-13	Mucus secretion
		IL-4	Th2 differentiation
		IL-5	Eosinophil production and activation
Eosinophils	Stored preformed in cytoplasmic granules	Major basic protein, eosinophil cationic protein	Toxic to helminths, bacteria, host cells
		Enzymes: eosinophil peroxidase, lysosomal hydrolases, lysophospholipase	Degradation of helminthic and protozoan cell walls; tissue damage/remodeling
	Preformed in cytosol	Galectin 10	Formation of Charcot-Leyden crystals; activation of inflammasomes, promoting inflammation and Th2 responses
	Major lipid mediators produced on activation	Leukotrienes C_4, D_4, E_4	Prolonged bronchoconstriction; mucus secretion; increased vascular permeability
	Cytokines produced on activation	IL-4	Th2 differentiation
		TGF-β	Fibrosis
		IL-8, IL-10, RANTES, MIP-1α, eotaxin	Chemotaxis of leukocytes

DC, Dendritic cell; *FcεRI,* Fcε receptor type I; *GM-CSF,* granulocyte-monocyte colony-stimulating factor; *IL,* interleukin; *MIP-1α,* monocyte inflammatory protein 1α; *PAF,* platelet-activating factor; *PGD_2*, prostaglandin D2; *RANTES,* regulated by activation, normal T cell expressed and secreted; *TNF,* tumor necrosis factor; *TGF-β,* transforming growth factor-β.

The importance of FcεRI in IgE-mediated immediate hypersensitivity reactions has been demonstrated in FcεRI *α* chain knockout mice. When these mice are given intravenous injections of IgE specific for a known antigen followed by that antigen, anaphylaxis does not develop or is mild, whereas it is a severe reaction in wild-type mice treated in the same way. FcεRI expression on the surface of mast cells and basophils is increased by IgE, thereby providing a mechanism for the amplification of IgE-mediated reactions.

Whereas FcεRI on mast cells and basophils is expressed as an $\alpha\beta\gamma_2$ tetramer, the receptors on eosinophils are mainly $\alpha\gamma_2$ trimers and are expressed at low levels, and there is no convincing evidence that eosinophils can be activated by antigens binding to IgE attached to FcεRI. Another IgE receptor called FcεRII, also known as CD23, is a protein related to C-type mammalian lectins whose affinity for IgE is much lower than that of FcεRI. The biologic role of FcεRII is not known.

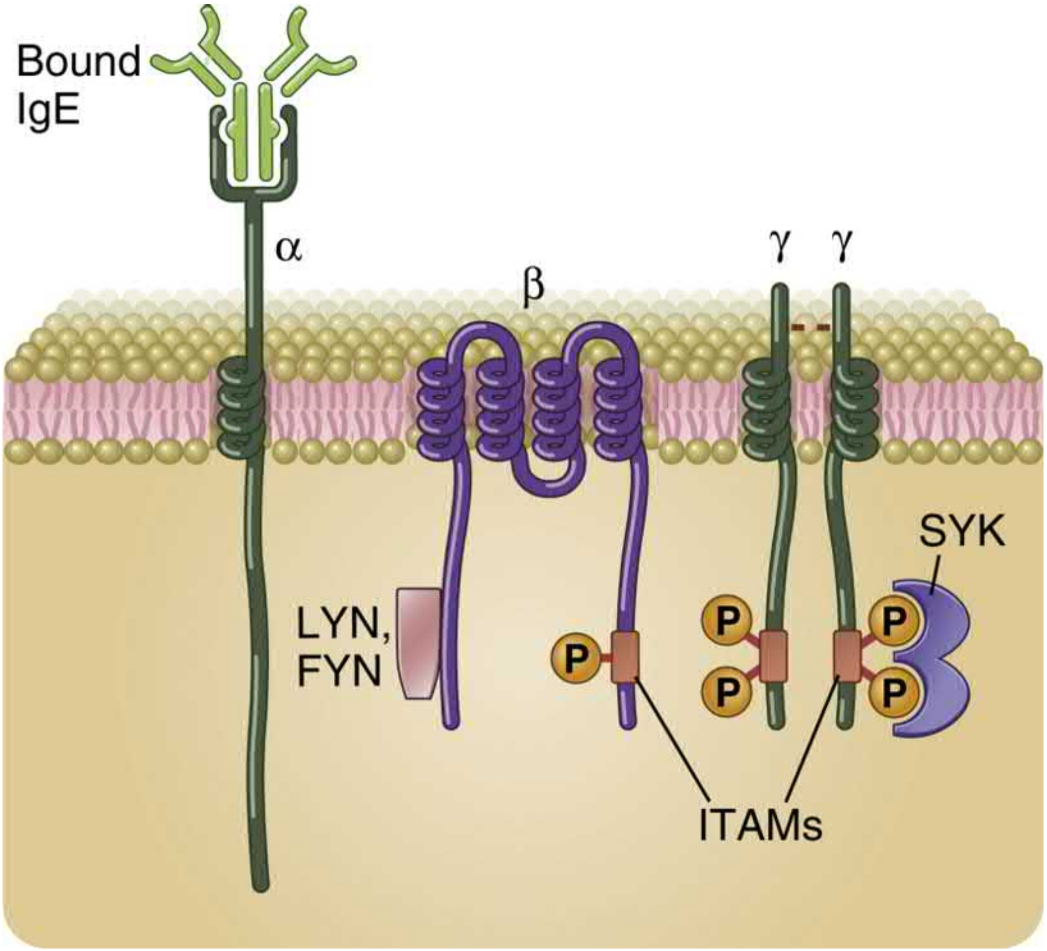


FIGURE 20.3 Polypeptide chain structure of the high-affinity IgE-specific Fc receptor (FcεRI).IgE binds to the Ig-like domains of the α chain. The β chain and the γ chains mediate signal transduction. The ITAMs in the cytoplasmic region of the β and γ chains are similar to those found in the T cell receptor complex (see Fig. 7.8). LYN and FYN are tyrosine kinases that bind to the N-terminal cytoplasmic end of the β chain, and SYK is a tyrosine kinase that binds to phosphorylated ITAM motifs in the C-terminal cytoplasmic tails of the β and γ chains. These kinases participate in signaling events that activate mast cells. *ITAM,* Immunoreceptor tyrosine activation motif.

Activation of Mast Cells

Mast cells are activated by cross-linking of FcεRI molecules, which occurs by binding of multivalent antigens to the IgE molecules that are attached to the Fc receptors (Fig. 20.4) **.** In an individual allergic to a particular antigen, a large proportion of the IgE bound to FcεRI on the surface of mast cells is specific for that antigen. Exposure to the antigen will cross-link sufficient IgE molecules to trigger mast cell activation. In contrast, in nonatopic individuals, the IgE molecules bound to mast cells are specific for many different antigens, all of which may have induced low levels of IgE production. Therefore, no single antigen will cross-link enough of the IgE molecules to cause mast cell activation.

Activation of mast cells results in three types of biologic responses: secretion of preformed granule contents by exocytosis (degranulation), synthesis and secretion of lipid mediators, and synthesis and secretion of cytokines. The signaling cascades initiated by allergen-mediated FcεRI cross-linking are similar to the proximal signaling events initiated by antigen binding to lymphocytes (Fig. 20.5; also see Chapter 7). When FcεR1 is cross-linked by an allergen binding to the attached IgE, the LYN tyrosine kinase, which is constitutively associated with the cytoplasmic tail of the FcεRI β chain, phosphorylates the nearby ITAMs in the cytoplasmic tails of FcεRI β and γ chains. The tyrosine kinase SYK is then recruited to the ITAMs of the γ chain, becomes activated, and phosphorylates and activates other proteins in the signaling cascade, including several adaptor molecules and enzymes that participate in the formation of multicomponent signaling complexes, as described in T cells. The complex includes phospholipase Cγ (PLCγ), which catalyzes phosphatidylinositol bisphosphate breakdown to yield inositol trisphosphate (IP3) and diacylglycerol (DAG), which in turn generate Ca^{++} and protein kinase C (PKC) signals, respectively (see Chapter 7). FYN, a tyrosine kinase that is also constitutively associated with the cytoplasmic tail of the FcεRI β chain, phosphorylates the docking protein GAB2, leading to PI3-kinase activation, which contributes to the generation of Ca^{++} and PKC signals. These signaling events lead to the three major responses:

- ***Degranulation.*** Activated PKC phosphorylates the myosin light chain component of actin-myosin complexes located beneath the plasma membrane, leading to disassembly of the complex. This allows cytoplasmic granules to come in contact with the plasma membrane. The mast cell granule membrane then fuses with the plasma membrane, a process that is mediated by members of the SNARE protein family, which are involved in many other membrane fusion events. Different SNARE proteins present on the granule membranes and plasma membranes interact to form a multimeric complex that catalyzes fusion. The formation of SNARE complexes is regulated by several accessory molecules, including RAB3 guanosine triphosphatases and RAB-associated kinases and phosphatases. In resting mast cells, these enzymes inhibit mast cell granule membrane fusion with the plasma membrane. On FcεRI cross-linking, the resulting increase in cytoplasmic calcium concentrations and the activation of PKC block the activity of the inhibitory molecules. In addition, calcium sensor proteins respond to the elevated calcium concentrations by promoting SNARE complex formation and membrane fusion. After membrane fusion, the contents of the mast cell granules are released into the extracellular environment. This process can occur within seconds of FcεRI cross-linking and can be visualized morphologically by loss of the dense granules of mast cells (see Fig. 20.4). The biologic actions of the granule contents released upon mast cell degranulation are described later.
- ***Lipid mediator production.*** Synthesis of lipid mediators is controlled by the cytosolic enzyme phospholipase A2 (PLA_2) (see Fig. 20.5). This enzyme is activated by two signals: elevated cytoplasmic Ca^{++} and phosphorylation

catalyzed by a MAP (mitogen-activated protein) kinase, such as ERK (extracellular receptor-activated kinase). ERK is activated as a consequence of a kinase cascade initiated through the FcεRI ITAMs, probably using the same intermediates as in T cells (see Chapter 7). Once activated, PLA_2 hydrolyzes membrane phospholipids to release arachidonic acid, which is converted by cyclooxygenase or lipoxygenase into different mediators (discussed later).

- ***Cytokine production.*** Cytokine secretion by activated mast cells is a consequence of newly induced cytokine gene transcription. The biochemical events that regulate cytokine gene transcription in mast cells appear to be similar to the events that occur in T cells. Recruitment and activation of various adaptor molecules and kinases in response to FcεRI cross-linking lead to nuclear translocation of NFAT (nuclear factor of activated T cells) and NF-κB (nuclear factor κB), as well as activation of AP1 (activator protein 1) by protein kinases such as c-JUN N-terminal kinase. These transcription factors stimulate expression of several cytokines (IL-4, IL-5, IL-6, IL-13, and tumor necrosis factor [TNF], among others) but, in contrast to T cells, not IL-2.

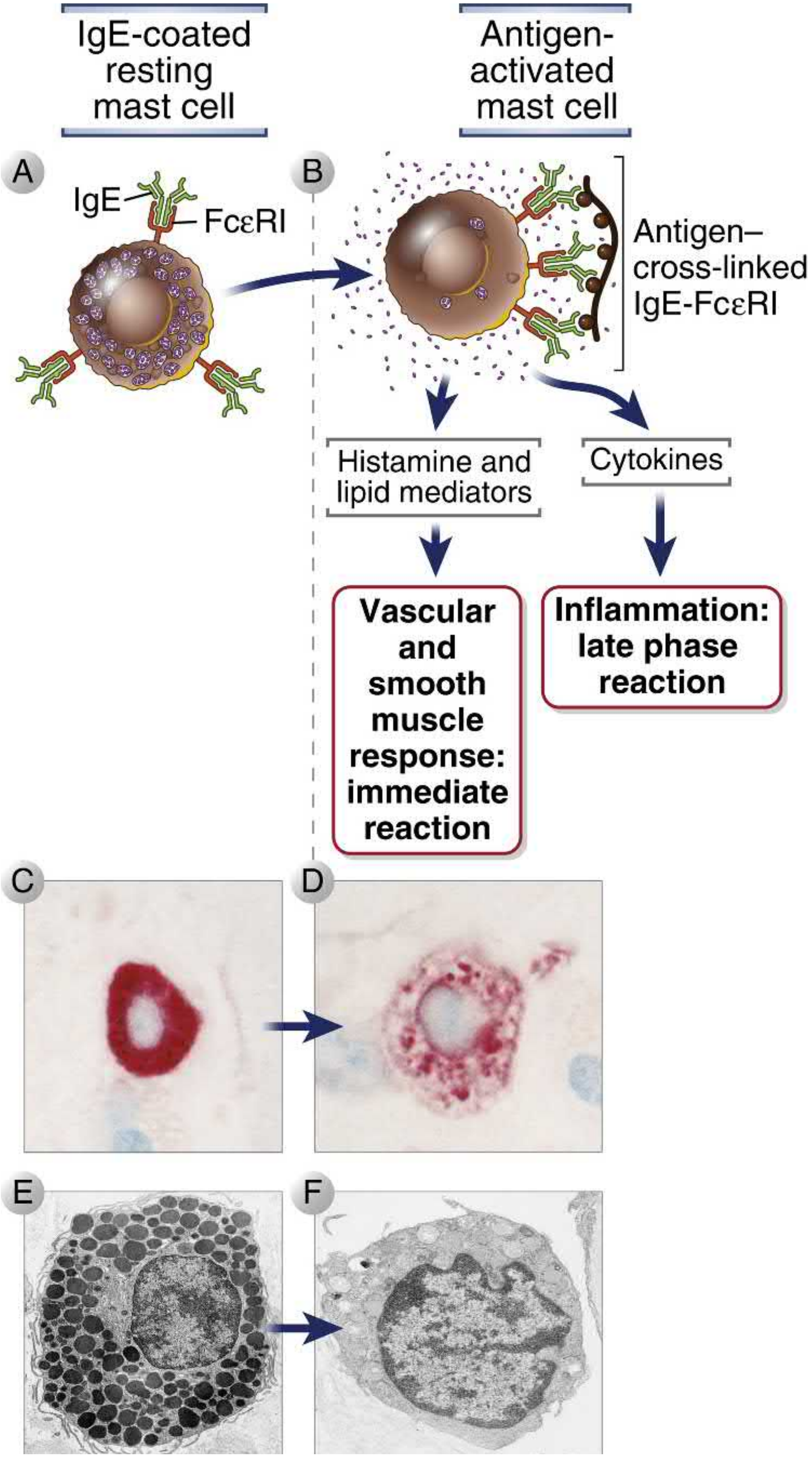

IgE-coated resting mast cell
Antigen-activated mast cell
A
IgE
FcεRI
B
Antigen–cross-linked IgE-FcεRI
Histamine and lipid mediators
Cytokines
Vascular and smooth muscle response: immediate reaction
Inflammation: late phase reaction
C
D
E
F

FIGURE 20.4 Mast cell activation.Antigen binding to immunoglobulin E IgE cross-links FcεRI molecules on mast cells, which induces the release of mediators that cause the hypersensitivity reaction (**A** and **B**). Other stimuli, including the complement fragment Toll-like receptor (TLR) ligands, the complement fragment C5a, cytokines, neuropeptides, and cationic secretagogue, can also activate mast cells independent of FcεRI. A photomicrograph of a resting mast cell with abundant *purple-staining* cytoplasmic granules is shown in **C.** These granules are also seen in the electron micrograph of a resting mast cell shown in **E.** In contrast, the depleted granules of an activated mast cell are shown in the photomicrograph **(D)** and electron micrograph **(F)**.

Courtesy Dr. Daniel Friend, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.

Mast cell activation through the FcεRI pathway is regulated by various inhibitory receptors, which contain immunoreceptor tyrosine-based inhibition motifs (ITIMs) within their cytoplasmic tails (see Chapter 7). One such inhibitory receptor is FcγRIIB, which co-aggregates with FcεRI during mast cell activation. The ITIM of FcγRIIB is phosphorylated by LYN, and this leads to recruitment of the phosphatase called SHIP (SH2 domain–containing inositol 5-phosphatase) and inhibition of FcεRI signaling. Experiments in mice indicate that FcγRIIB inhibits mast cell degranulation in vivo. Several other inhibitory receptors are also expressed on mast cells, but their importance in vivo is not known.

In addition to allergen-induced cross-linking of FcεRI, many other inflammatory stimuli can activate mast cells in the absence of allergens or synergize with allergens. The complement fragments C3a and C5a can cause mast cell degranulation, and this is why they were named anaphylatoxins. Other stimuli induce selective activation of mast cells to produce arachidonic acid metabolites, cytokines, and chemokines, but not degranulation. These stimuli include Toll-like receptor (TLR) ligands; substances released from injured cells, including released ATP; fungal glucans; antimicrobial peptides; cytokines such as stem cell factor (SCF), IL-3, IL-4, IL-9, and IL-33; leukotrienes; and several chemokines. These additional IgE-independent modes of mast cell activation are likely important for the physiologic role of mast cells as sentinel cells of innate immunity, initiating inflammatory responses to infection or tissue injury (see Chapter 4).

Many neuropeptides, including substance P, somatostatin, and vasoactive intestinal peptide, induce mast cell histamine release and may mediate neuroendocrine-linked mast cell activation. The nervous system is known to modulate allergic reactions, and neuropeptides may be involved in this effect. The flare produced at the edge of the wheal in elicited immediate hypersensitivity reactions is in part mediated by the nervous system, as shown by the observation that it is markedly diminished in skin sites lacking innervation. Cold temperatures and intense exercise also trigger mast cell degranulation, but the mechanisms involved are not known.

Mast cell degranulation also can be stimulated by many different cationic substances, collectively called secretagogues. These include endogenous inflammatory peptides, drugs known to cause adverse allergy-like reactions, and the compounds 48/80 and mastoparan used experimentally as pharmacologic triggers for mast cells.

Many agents activate mast cells independent of FcεRI by binding to a receptor called MAS-related G protein–coupled receptor-X2 (MRGPRX2), which is highly expressed only by skin and other tissue mast cells and neurons of the dorsal root ganglion, but not mucosal mast cells. Ligands that bind to MRGPRX2 and thereby activate mast cells include several antibiotics and anesthetic drugs, components of insect venoms, antimicrobial peptides, molecules secreted by eosinophil and neuropeptides. MRGPRX2 is considered to be a likely mediator of many allergy-like reactions to drugs and other pathologic conditions characterized by urticaria (superficial dermal edema with itching).

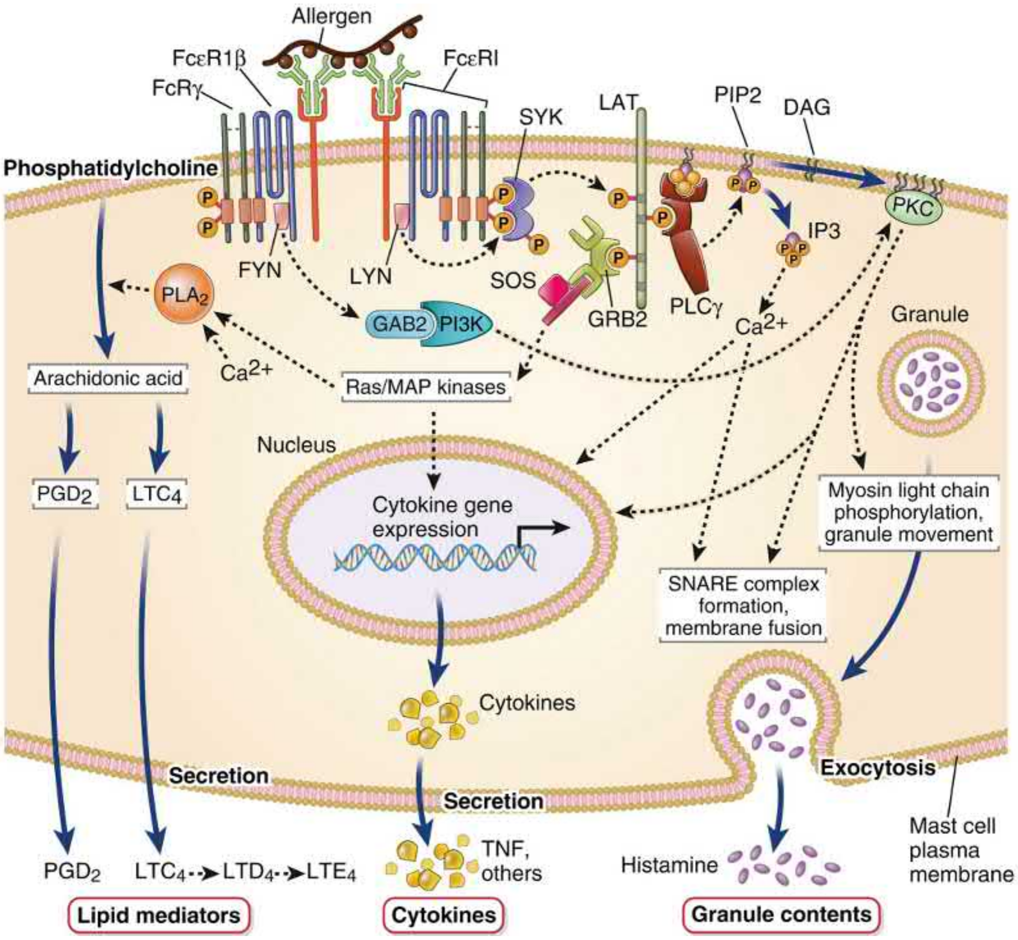

Allergen
FcεR1β
FcεRI
FcRγ
SYK
LAT
PIP2
DAG
Phosphatidylcholine
P
PKC
IP3
FYN
LYN
SOS
GRB2
PLCγ
Ca^{2+}
Granule
PLA$_2$
GAB2
PI3K
Arachidonic acid
Ca^{2+}
Ras/MAP kinases
Nucleus
PGD$_2$
LTC$_4$
Cytokine gene expression
Myosin light chain phosphorylation, granule movement
SNARE complex formation, membrane fusion
Cytokines
Secretion
Secretion
Exocytosis
Mast cell plasma membrane
PGD$_2$
LTC$_4$ → LTD$_4$ → LTE$_4$
TNF, others
Histamine
Lipid mediators
Cytokines
Granule contents

FIGURE 20.5 Biochemical events of mast cell activation.Cross-linking of bound immunoglobulin E by antigen promotes LYN phosphorylation of other signaling molecules, which leads to activation of protein tyrosine kinase SYK, which in turn causes activation of a mitogen-activated protein *(MAP)* kinase cascade and phospholipase Cγ *(PLCγ).* PLCγ catalyzes the release of inositol trisphosphate *(IP3)* and diacylglycerol *(DAG)* from membrane phosphatidylinositol 4,5-bisphosphate *(PIP2).* IP3 causes release of intracellular calcium from the endoplasmic reticulum. Calcium and DAG activate protein kinase C *(PKC).* FYN phosphorylation of GAB2 leads to PI3K activation, which contributes to PKC activation. Calcium, MAP kinases, and PKC promote cytokine gene

transcription, leading to secretion of cytokines. PKC and calcium also enhance granule exocytosis, releasing histamine and other preformed mediators. Calcium and MAP kinases combine to activate the enzyme cytosolic phospholipase A2 *(PLA $_2$),* which initiates the synthesis of lipid mediators, including prostaglandin D_2 *(PGD $_2$)* and leukotriene C_4 *(LTC $_4$).*

Mast cells also express Fc receptors for IgG heavy chains, and the cells can be activated by cross-linking bound IgG. This IgG-mediated reaction is the likely explanation for the finding that Ig ε chain knockout mice are not completely resistant to antigen-induced mast cell–mediated anaphylaxis. However, IgE is the major antibody isotype involved in most immediate hypersensitivity reactions.

Mast cell activation is not an all-or-nothing phenomenon, and different types or levels of stimuli may elicit partial responses, with production of some mediators but not others. Such variations in activation and mediator release may account for variable clinical presentations.

Mediators Derived From Mast Cells

The effector functions of mast cells are mediated by soluble molecules released from the activated cells (Fig. 20.6; see also Table 20.2) **.** These mediators may be divided into preformed mediators, which include vasoactive amines, and newly synthesized mediators, which include lipid mediators and cytokines.

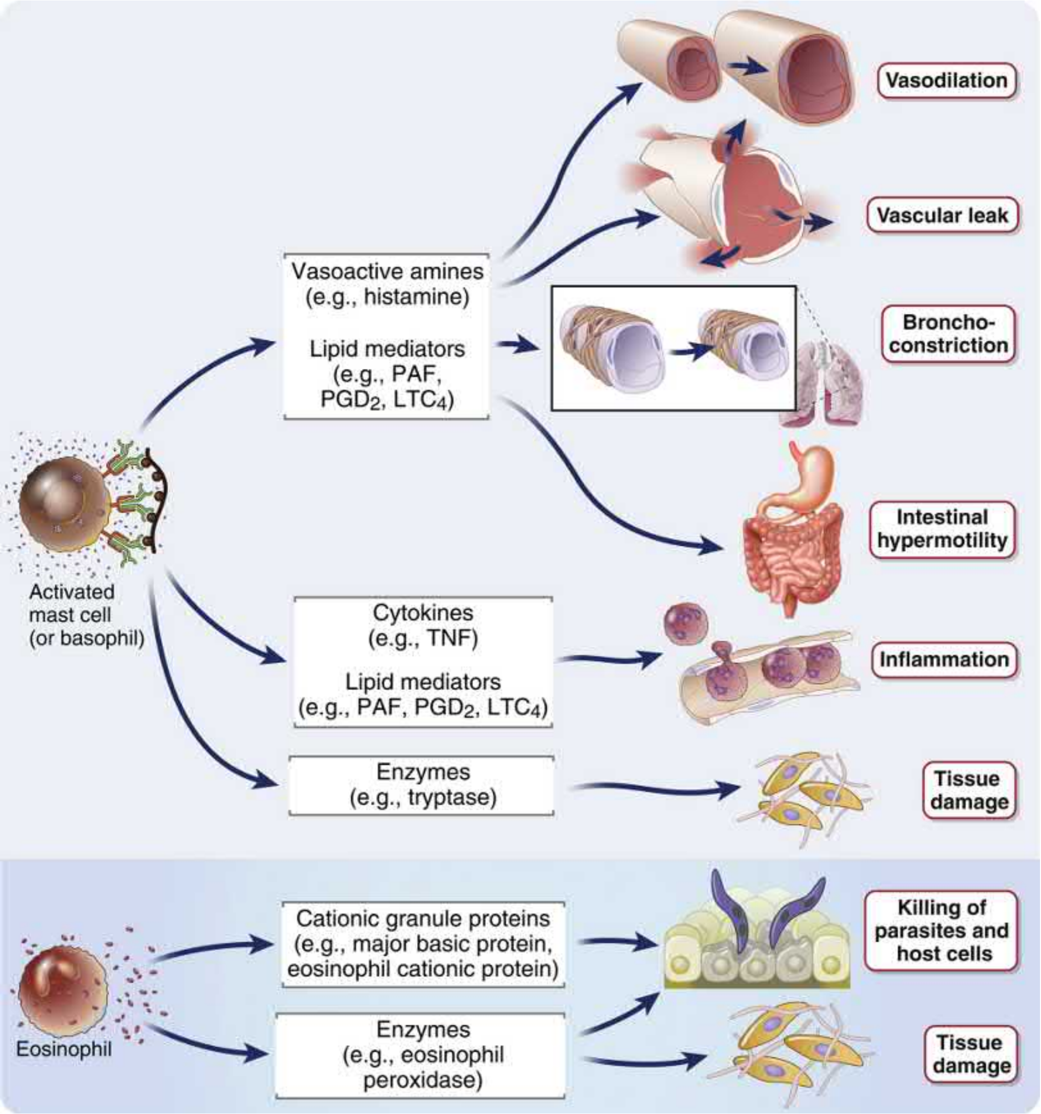

Vasodilation
Vascular leak
Vasoactive amines (e.g., histamine)
Lipid mediators (e.g., PAF, PGD_2, LTC_4)
Broncho-constriction
Intestinal hypermotility
Activated mast cell (or basophil)
Cytokines (e.g., TNF)
Lipid mediators (e.g., PAF, PGD_2, LTC_4)
Inflammation
Enzymes (e.g., tryptase)
Tissue damage
Cationic granule proteins (e.g., major basic protein, eosinophil cationic protein)
Killing of parasites and host cells
Eosinophil
Enzymes (e.g., eosinophil peroxidase)
Tissue damage

FIGURE 20.6 Biologic effects of mediators of immediate hypersensitivity.Mast cell and basophil mediators include vasoactive amines and enzymes stored preformed in granules, as well as cytokines and lipid mediators, which are largely newly synthesized on cell activation. The biogenic amines and lipid mediators induce vascular leakage, bronchoconstriction, and intestinal hypermotility, all components of the immediate response. Cytokines and lipid mediators contribute to inflammation, which is part of the late-phase reaction. Enzymes probably contribute

to tissue damage. Activated eosinophils release preformed cationic proteins and enzymes that are toxic to parasites and host cells. Some eosinophil granule enzymes probably contribute to tissue damage in chronic allergic diseases. *LTC_4*, Leukotriene C_4; *PAF*, platelet-activating factor; *PGD_2*, prostaglandin D_2; *TNF*, tumor necrosis factor.

Vasoactive Amines

Many of the biologic effects of mast cell activation are mediated by vasoactive amines that are released from cytoplasmic granules and act on blood vessels and smooth muscle. Vasoactive amines are low-molecular-weight compounds that contain an amine group and act directly on blood vessels. In human mast cells, the major mediator of this class is **histamine,** but in some rodents, serotonin may also be important. Histamine acts by binding to target cell receptors, and different cell types express distinct classes of histamine receptors (e.g., H1, H2, H3) that can be distinguished by their sensitivity to different pharmacologic inhibitors. The actions of histamine are short-lived because histamine is rapidly removed from the extracellular milieu by amine-specific transport systems. Histamine binding to cellular receptors initiates intracellular events, such as phosphatidylinositol breakdown to IP3 and DAG, and these products cause different changes in different cell types. Histamine actions on endothelium include contraction of the endothelial cells, leading to increased interendothelial spaces, increased vascular permeability, and leakage of plasma into the tissues. Histamine also stimulates endothelial cells to synthesize vascular smooth muscle cell relaxants, such as prostacyclin (PGI_2) and nitric oxide, which cause vasodilation. These actions of histamine produce the wheal-and-flare response of immediate hypersensitivity (described later). H1 receptor antagonists (commonly called antihistamines) can inhibit the vascular responses to intradermal allergen. Histamine also causes contraction of intestinal and bronchial smooth muscle. Thus, histamine may contribute to the increased peristalsis and bronchospasm associated with ingested and inhaled allergens, respectively. However, in some allergic disorders, and especially in asthma, antihistamines are not effective at suppressing the reaction. Moreover, bronchoconstriction in asthma is more prolonged than are the effects of histamine, indicating that other mast cell–derived mediators are important in some forms of allergy.

Granule Enzymes and Proteoglycans

Neutral serine proteases, including tryptase and chymase, are the most abundant protein constituents of mast cell secretory granules and may contribute to tissue damage in immediate hypersensitivity reactions. Tryptase is present in all human mast cells and is not known to be present in any other cell type. Therefore, the presence of tryptase in human biologic fluids is interpreted as a marker of mast cell activation, and serum tryptase assays have been used to diagnose anaphylaxis and other disorders associated with mast cell activation. Chymase is found in some human mast cells, and its presence or absence is one criterion for characterizing human mast cell subsets, as discussed earlier. The functions of these enzymes in vivo are not established; however, several activities demonstrated in vitro suggest important biologic actions. For example, tryptase cleaves and activates collagenase, thereby causing tissue damage, whereas chymase can convert angiotensin I to angiotensin II, which causes transient vasoconstriction. Chymase also degrades epidermal basement membranes and stimulates mucus secretion. Other enzymes found within mast cell granules include carboxypeptidase A and cathepsin G. Basophil granules also contain several enzymes, some of which are the same as those in mast cell granules, such as neutral proteases.

Proteoglycans, including heparin and chondroitin sulfate, are also major constituents of mast cell and basophil granules. These molecules are composed of a polypeptide core and multiple unbranched glycosaminoglycan side chains that impart a strong net negative charge to the molecules. Within the granules, proteoglycans serve as storage matrices for positively charged amines, proteases, and other mediators and prevent their accessibility to the rest of the cell. The mediators are released from the proteoglycans at different rates after granule exocytosis, with vasoactive amines dissociating more rapidly than tryptase or chymase. In this way, the proteoglycans may control the kinetics of immediate hypersensitivity reactions.

Lipid Mediators

Mast cell activation results in the rapid de novo synthesis and release of lipid mediators that have a variety of effects on blood vessels, bronchial smooth muscle, and leukocytes. The most important of these mediators are derived from arachidonic acid, which is generated by PLA_2-mediated hydrolysis of membrane phospholipids, as discussed earlier. Arachidonic acid is then metabolized by either the cyclooxygenase or lipoxygenase pathways to produce mediators of allergic reactions.

The major arachidonic acid–derived mediator produced by the cyclooxygenase pathway in mast cells is **prostaglandin D2** (PGD_2). Released PGD_2 binds to receptors on smooth muscle cells and acts as a vasodilator and a bronchoconstrictor. PGD_2 also promotes neutrophil chemotaxis and accumulation at inflammatory sites. PGD_2 synthesis can be prevented by cyclooxygenase inhibitors, such as aspirin and other nonsteroidal antiinflammatory agents. These drugs may paradoxically exacerbate asthmatic bronchoconstriction because they shunt arachidonic acid toward production of leukotrienes, discussed next.

The major arachidonic acid–derived mediators produced by the lipoxygenase pathway are the **leukotrienes,** especially LTC_4 and its degradation products LTD_4 and LTE_4, all of which are called cysteinyl leukotrienes. LTC_4 is made mainly by mast cells in mucosa and by basophils, but not by mast cells in connective tissues. Mast cell–derived leukotrienes bind to specific receptors on smooth muscle cells, different from the receptors for PGD_2, and cause prolonged bronchoconstriction. Collectively, the cysteinyl leukotrienes constitute what was once called slow-reacting substance of anaphylaxis (SRS-A) and are now known to be important mediators of bronchoconstriction in asthma. When injected into the skin, these leukotrienes produce a long-lived wheal-and-flare reaction.

A third type of lipid mediator produced by mast cells and basophils, as well as several other cell types, is platelet-activating factor (PAF), named for its discovery as an inducer of rabbit platelet aggregation. PAF is synthesized as a derivative of membrane phospholipids. It has direct bronchoconstricting actions, causes retraction of endothelial cells, and relaxes vascular smooth muscle. However, PAF is hydrophobic and is rapidly destroyed by a plasma enzyme called PAF hydrolase, which limits its biologic actions. Individuals with an inherited deficiency of PAF hydrolase are at high risk for developing early-onset asthma. Levels of PAF and its metabolites are elevated in anaphylaxis. In rodent models, pharmacologic inhibitors of PAF receptors ameliorate some aspects of immediate hypersensitivity in the lung, but PAF antagonists have not proved useful in clinical trials. PAF also may be important in late-phase reactions, in which it can activate inflammatory leukocytes.

Cytokines

Mast cells produce many cytokines that contribute to allergic inflammation (the late-phase reaction). These cytokines include TNF, IL-1, IL-4, IL-5, IL-6, IL-9, IL-13, CCL3, CCL4, and various colony-stimulating factors, such as IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). As mentioned earlier, mast cell activation induces transcription and synthesis of these cytokines. Th2 cells that are recruited into the sites of allergic reactions also produce some of these cytokines. The cytokines that are released from activated mast cells, Th2 cells, and possibly ILC2s are mainly responsible for the inflammation associated with the late-phase reaction. TNF activates endothelial expression of adhesion molecules and together with chemokines accounts for neutrophil and monocyte recruitment to the reaction site (see Chapter 3). In addition to allergic inflammation, mast cell cytokines also contribute to innate immune responses to infections. For example, as we will discuss later, mouse models indicate that mast cells are required for effective defense against some bacterial infections, and this effector function is mediated largely by TNF.

Properties of Eosinophils

Eosinophils are bone marrow–derived granulocytes that are abundant in the inflammatory infiltrates of late-phase reactions and are involved in many of the pathologic processes in allergic diseases. GM-CSF, IL-3, and IL-5 promote eosinophil differentiation from myeloid precursors in the bone marrow, and after maturation they circulate in the blood. Eosinophils are normally present in peripheral tissues, especially in mucosal linings of the respiratory, gastrointestinal, and genitourinary tracts. The granules of eosinophils contain basic proteins that bind acidic dyes such as eosin (see Table 20.2 and Fig. 20.2C). Eosinophils express very low levels of FcεRI, and the receptor lacks a signaling chain, so its function in these cells is unclear.

Cytokines produced by Th2 cells and ILC2s promote the activation of eosinophils and their recruitment to late-phase reaction sites. Both Th2 cells and ILC2s are sources of IL-5. IL-5 stimulates bone marrow production of eosinophils and is a potent activator of mature eosinophils that enhances the ability of these cells to release granule contents. In the absence of this cytokine (e.g., in IL-5 knockout mice), there is a deficiency of eosinophil numbers and functions. Asthma patients treated with anti–IL-5 or anti–IL-5 receptor antibodies also show decreased eosinophil numbers and amelioration of symptoms. Eosinophils are recruited into late-phase reaction sites, as well as sites of helminthic infection, and their recruitment is mediated by a combination of adhesion molecule interactions and chemokines. Eosinophils bind to endothelial cells expressing E-selectin and VCAM-1, the ligand for the VLA-4 integrin. IL-4 produced by Th2 cells may enhance expression of adhesion molecules for eosinophils. Eosinophil recruitment and infiltration into tissues also depend on the chemokine eotaxin (CCL11), which is produced by epithelial cells at sites of allergic reactions and binds to the chemokine receptor CCR3, which is expressed on eosinophils. In addition, the complement product C5a and the lipid mediators PAF and LTB_4 produced by mast cells also function as chemoattractants for eosinophils.

Upon activation, eosinophils release granule proteins that are toxic to microbes and may injure normal tissues. The granule contents of eosinophils include lysosomal hydrolases found in other granulocytes as well as eosinophil-specific proteins that are particularly toxic to helminthic organisms, including major basic protein and eosinophil cationic protein. These two cationic polypeptides have no known enzymatic activities, but they damage the integument of helminths and the cell walls of bacteria, as well as cells in normal tissues. In addition, eosinophilic granules contain eosinophil peroxidase, which is distinct from the myeloperoxidase found in neutrophils and catalyzes the production of hypochlorous or hypobromous acid. These products are also toxic to helminths, protozoa, and host cells.

Activated eosinophils, like mast cells and basophils, produce and release lipid mediators, including PAF, prostaglandins, and the cysteinyl leukotrienes. These eosinophil-derived lipid mediators may contribute to the pathologic processes of allergic diseases. Eosinophils also produce a variety of cytokines, including IL-4 and TGF-β, which may promote inflammatory responses and fibrosis receptively. Eosinophils produce large amounts of the protein galectin-10 compared to other cell types, and when released from activated and dying eosinophils, the galectin 10 aggregates to form crystals, historically called Charcot-Leyden crystals. These crystals are often found in sites of chronic type 2 inflammation, such as the bronchi of allergic asthma patients. Experimental evidence indicates that Charcot-Leyden crystals have a variety of proinflammatory activities, including inflammasome activation and priming of DCs to induce Th2 cell differentiation. These effects are reduced in experimental animals by treatment with antibodies that specially block crystallization of galectin 10.

Reactions Dependent on IgE and Mast Cells

The cells and mediators we have discussed are responsible for the immediate vascular changes and the later inflammatory reactions that occur in allergies (Fig. 20.7). In the following sections, we will describe these immediate and late-phase reactions.

The Immediate Reaction

The early vascular changes that occur during immediate hypersensitivity reactions are demonstrated by the wheal-and-flare reaction to intradermal injection of an allergen (Fig. 20.8)**.** When an individual who has previously encountered an allergen and produced IgE antibody is challenged by intradermal injection of the same antigen, the injection site becomes red from locally dilated blood vessels engorged with red blood cells. The site then rapidly swells as a result of leakage of plasma from the venules. This soft swelling is called a wheal and can involve an area of skin as large as several centimeters in diameter. Subsequently, blood vessels at the margins of the wheal dilate and become engorged with red blood cells, producing a characteristic red rim called a flare. The full wheal-and-flare reaction can appear within 5 to 10 minutes after administration of antigen and usually subsides in less than 1 hour.

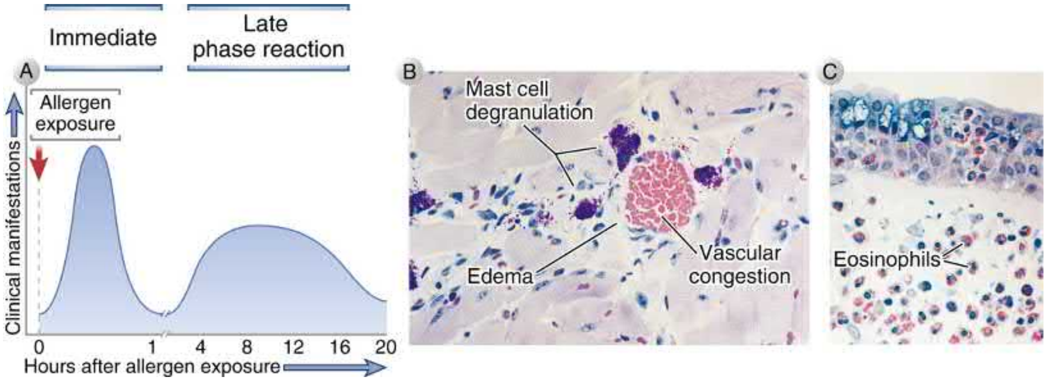

Immediate
Late phase reaction
A
Allergen exposure
Clinical manifestations
0
1
4
8
12
16
20
Hours after allergen exposure
B
Mast cell degranulation
Edema
Vascular congestion
C
Eosinophils

FIGURE 20.7 The immediate and late-phase reactions of allergy. **A,** Kinetics. The immediate vascular and smooth muscle reaction to allergen develops within minutes after challenge (allergen exposure in a previously sensitized individual), and the late-phase reaction develops 2 to 24 hours later. **B** and **C,** Morphology. The immediate reaction **(B)** is characterized by vasodilation, congestion, and edema, and the late-phase reaction **(C)** is characterized by an inflammatory infiltrate rich in eosinophils, neutrophils, and T cells.

Courtesy of the late Dr. Daniel Friend, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.

The wheal-and-flare reaction is dependent on IgE and mast cells. Histologic examination shows that mast cells in the area of the wheal-and-flare have discharged their cytoplasmic granules (i.e., released their preformed mediators). A causal association of IgE and mast cells with immediate hypersensitivity was first deduced from experiments involving the passive transfer of IgE antibodies from an allergic individual into a normal recipient. For example, immediate hypersensitivity reactions against an allergen can be elicited in unresponsive individuals if the local skin site is first injected with IgE from an allergic individual. Such adoptive transfer experiments were first performed with serum from immunized individuals, and the serum factor responsible for the reaction was originally called reagin. For this reason, IgE molecules are still sometimes called reaginic antibodies. Subsequent experiments showed that a wheal and flare response could be reproduced by injection of anti-IgE antibodies that cross-link mast cell FcεRI to which IgE is attached. The antigen-initiated skin reaction that follows adoptive transfer of IgE is called passive cutaneous anaphylaxis.

The wheal-and-flare reaction results from sensitization of dermal mast cells by IgE binding to FcεRI, cross-linking of IgE by the antigen, and activation of mast cells with release of mediators, notably histamine. Histamine binds to histamine receptors on venular endothelial cells; the endothelial cells synthesize and release PGI_2 and nitric oxide, and these mediators cause vasodilation and vascular leak, as described earlier. Skin mast cells appear to produce only small amounts of long-acting mediators such as leukotrienes, so the wheal-and-flare response subsides rapidly. Allergists often test patients for allergies to different antigens by examining the ability of these antigens applied in skin patches or administered through small needle pricks to elicit wheal-and-flare reactions.

The Late-Phase Reaction

The immediate wheal-and-flare reaction is followed 2 to 4 hours later by a late-phase reaction consisting of the accumulation of inflammatory leukocytes, including neutrophils, eosinophils, basophils, and helper T cells (see Fig. 20.7) **.** The inflammation is maximal by about 24 hours and then gradually subsides. Like the immediate wheal-and-flare reaction, the capacity to mount a late-phase reaction also can also be adoptively transferred with IgE, and the reaction can be mimicked with anti-IgE antibodies or with mast cell–activating agents. Cytokines produced by mast cells, including TNF, upregulate endothelial expression of leukocyte adhesion molecules, such as E-selectin and ICAM-1 (intercellular adhesion molecule 1), and chemokines, which results in the recruitment of blood leukocytes (see Chapter 3). Thus, mast cell activation promotes the influx of leukocytes into tissues. The types of leukocytes that are typical of late-phase reactions are eosinophils and helper T cells. Although Th2 cells are the dominant T cell population in uncomplicated late-phase reactions, the cellular infiltrates in chronic atopic dermatitis and asthma contain Th1 and Th17 cells, as well as T cells that produce both IL-17 and IFN-γ. Neutrophils are also often present in these reactions. Eosinophils and Th2 cells both express CCR4 and CCR3, and the chemokines that bind to these receptors are produced by many cell types at sites of immediate hypersensitivity reactions, including epithelial cells.

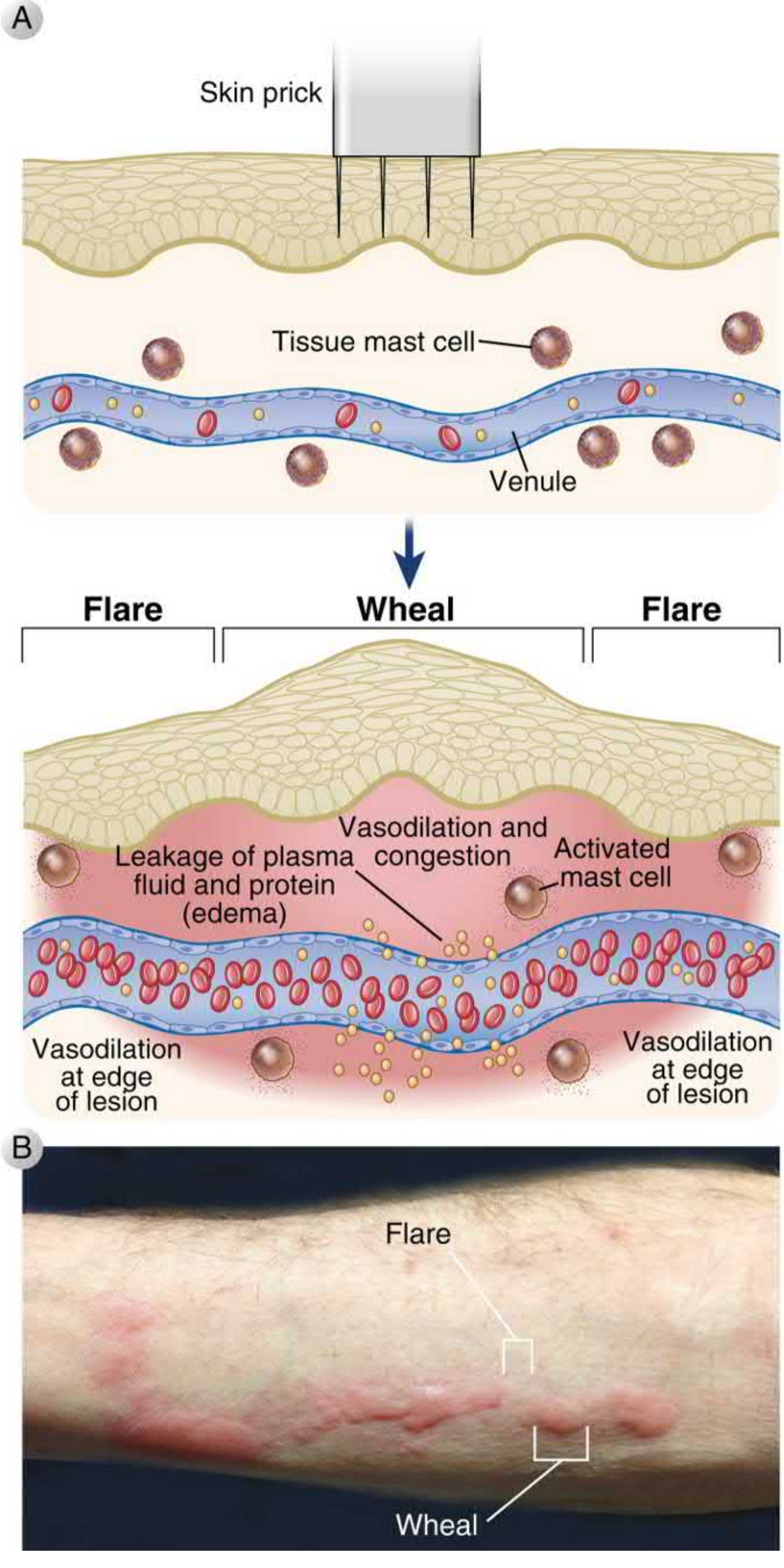

A
Skin prick
Tissue mast cell
Venule
Flare
Wheal
Flare
Vasodilation and congestion
Leakage of plasma fluid and protein (edema)
Activated mast cell
Vasodilation at edge of lesion
Vasodilation at edge of lesion
B
Flare
Wheal

FIGURE 20.8 The wheal-and-flare reaction in the skin and allergy skin tests. **A,** In a clinical test for allergies, different antigens are introduced into the skin by short needles. Patients with allergies to an antigen will have antigen-specific IgE already bound to mast cells in the skin, and the mast cells will be activated. In response to antigen-stimulated release of mast cell mediators, local blood vessels first dilate and then become leaky to fluid and macromolecules, which produces redness and local swelling (a wheal). Subsequent dilation of vessels on the edge of the swelling produces the appearance of a red rim (the flare). **B,** Photograph of a typical positive skin test showing wheal-and-flare reactions in the skin in response to injection of allergens.

Courtesy Dr. David Sloane, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.

The late-phase reaction may occur without a detectable preceding immediate hypersensitivity reaction. Bronchial asthma is a disease in which there may be repeated bouts of inflammation with accumulations of eosinophils and Th2 cells without the vascular changes that are characteristic of the immediate response. In such disorders, there may be little mast cell activation, and the cytokines that sustain the late-phase reaction may be produced mainly by T cells.

Genetic Susceptibility to Allergic Disease

The propensity to develop allergies is influenced by the inheritance of several genes. Atopic disease often affects several members of the same family, and studies have shown autosomal transmission of atopy, but the full inheritance pattern is multigenic. Within the same family, the target organ of atopic disease is variable. Thus, allergic rhinitis (hay fever), asthma, and atopic dermatitis (eczema) can be present to various degrees in different members of the same kindred and may appear at different times. All these individuals may show higher than average plasma IgE levels.

Various approaches have been taken to identify genes that carry a risk for allergic diseases, including positional cloning, candidate gene studies, and genome-wide association studies. These approaches have identified many different gene variants that confer increased susceptibility for asthma and other atopic diseases (Table 20.3). Based on the known functions of the proteins encoded by many of these genes, rational speculations can be made about how altered expression or activity of these proteins might affect the development or severity of allergic diseases. Nonetheless, we still know very little about whether the genetic polymorphisms that are associated with increased risk for allergy actually alter expression or function of the encoded proteins, and, in many cases, it is not clear how the function of many of the encoded proteins could affect the development of allergy.

One of the first significant findings from genetic studies of allergy was the identification of a susceptibility locus for atopy on chromosome 5q, near the site of the gene cluster encoding the cytokines IL-4, IL-5, IL-9, and IL-13. This region is of great interest because of the connection between several genes located there and the mechanisms of IgE regulation and mast cell and eosinophil growth and differentiation. Among the genes in this cluster, polymorphisms in the *IL33* gene appear to have the strongest association with asthma. The loci containing genes encoding IL33, a component of the IL33 receptor, and the transcription factor RORα have been identified in genome-wide association studies of asthma susceptibility. As discussed earlier, IL-33 is a cytokine released by damaged epithelial cells and is an inducer of type 2 inflammation, in which Th2 cells and ILC2s release IL-5 and IL-13. RORα may be required for ILC2 differentiation.

Mutations that result in loss of expression or function of the protein filaggrin result in significant risk for development of atopic dermatitis in early childhood, and subsequent allergic diseases, including asthma. As mentioned earlier, filaggrin is required for skin barrier functions and water retention, and a lack of this protein is thought to promote keratinocyte damage and cytokine release, as well as allergen entry into the dermis. Thus, filaggrin mutations may enhance risk for allergic disease by increasing the access of allergens to the immune system.

Table 20.3

Candidate Genes or Encoded Protein	Chromosomal Location	Disease Association	Postulated Role of Gene Products in Disease
Genes in cytokine gene cluster (IL-4, IL-5, IL-13), CD14, β_2-adrenergic receptor	5q	Asthma	IL-4 and IL-13 promote IgE switching, IL-5 promotes eosinophil growth and activation; CD14 is a component of the LPS receptor that, through interaction with TLR4, may influence the balance between Th1 and Th2 responses to antigens; β_2-adrenergic receptor regulates bronchial smooth muscle contraction
Class II MHC	6p	Asthma	Some alleles may regulate T cell responses to allergens
FcεRI β chain	11q	Asthma	Mediates mast cell activation
Stem cell factor, IFN-γ, STAT6	12q	Asthma	Stem cell factor regulates mast cell growth and differentiation; IFN-γ opposes actions of IL-4; STAT6 mediates IL-4 signal transduction
IL-4 receptor α chain	16	Asthma	Subunit of both IL-4 and IL-13 receptors
ADAM33	20p	Asthma	Metalloproteinase involved in airway remodeling
Filaggrin	1q	Atopic dermatitis	Component of terminally differentiated keratinocytes important for epithelial barrier function
IL-33, IL-33 receptor	2q	Asthma	IL-33 induces type 2 cytokines in T cells, mast cells, eosinophils, ILCs
ORMDL3	17q	Asthma	ER stress response
Phosphodiesterase 4D	5q	Asthma	Degrades cAMP and regulates airway smooth muscle contractility
TSLP	5q	Asthma	Activation of dermal dendritic cells

ADAM33, Disintegrin and metalloprotease domain 33; *cAMP,* cyclic adenosine monophosphate; *ER,* endoplasmic reticulum; *FcεRI,* Fcε receptor type I; *IFN-γ,* interferon- γ; *Ig,* immunoglobulin; *IL,* interleukin; *ILCs,* innate lymphoid cells; *LPS,* lipopolysaccharide; *MHC,* major histocompatibility complex; *ORMDL3,* orosomucoid like 3; *PHF11,* plant homeodomain finger protein 11; *STAT6,* signal transducers and activators of transcription-6; *TLR,* Toll-like receptors; *TSLP,* Thymic stromal lymphopoietin.

Some genes whose products regulate the innate immune response to infections have been associated with allergy and asthma. These include CD14, a component of the lipopolysaccharide receptor, and TLR2 and TLR4. It is possible that polymorphisms or mutations in genes that result in enhanced or diminished innate responses to common infectious organisms may influence the risk for development of atopy. Other genome-wide association studies have found significant associations of common variants of numerous other genes with asthma and other atopic diseases. However, either the products of these genes are of unknown function, or the connection between their known functions and the development of atopic disease is not known.

Environmental Factors in Allergy

It is clear that environmental influences have a significant impact on the development of allergy, and they synergize with genetic risk factors. Environmental influences include exposure to allergens themselves, to infectious organisms, and possibly other factors that have an impact on mucosal barrier function, such as air pollution. Furthermore, the time of life when exposure to these environmental factors occurs, especially early-life exposure, appears to be important.

Exposure to microbes during early childhood may reduce the risk for developing allergies. One possible explanation for the increased prevalence of asthma and other atopic diseases in industrialized countries is that the frequency of infections or exposure to microbial products in these countries is generally lower. A variety of epidemiologic data show that early childhood exposure to environmental microbes, such as those found on farms but not in cities, is associated with decreased prevalence of allergic disease. Based on these data, the **hygiene hypothesis** was proposed, which states that early-life and even perinatal exposure to environmental and commensal microbes and infections leads to a regulated maturation of the immune system and perhaps early development of regulatory T cells. As a result, later in life these individuals are less likely to mount Th2 responses to noninfectious environmental antigens and less likely to develop allergic diseases.

Respiratory viral and bacterial infections are a predisposing factor in the development of asthma and exacerbations of preexisting asthma. It is estimated that respiratory viral infections precede up to 80% of asthma attacks in children. This may seem contradictory to the hygiene hypothesis, but these asthma-associated infections are due to human pathogens that may damage pulmonary mucosal barriers; the data supporting the hygiene hypothesis focus on exposure to a broad range of environmental bacteria not necessarily related to tissue injury. Some epidemiologic studies indicate that a failure to colonize the respiratory or gastrointestinal tract early in life by particular commensal microbes can increase the risk for respiratory viral infections that induce asthma.

Allergic Diseases in Humans: Pathogenesis and Therapy

The manifestations of allergic diseases depend on the tissues in which the mast cell mediators and type 2 cytokines have effects, as well as the chronicity of the resulting inflammatory process. Atopic individuals may have one or more types of allergy, the most common forms being allergic rhinitis, bronchial asthma, atopic dermatitis, and food allergies. Frequently, an individual will develop more than one atopic disorder. The clinical presentation of atopic dermatitis in babies followed later in childhood by allergic rhinitis and asthma is known as the atopic march, and these three conditions, described later, are together called the atopic triad.

The clinical and pathologic features of allergic reactions vary with the anatomic site of the reaction, for several reasons. The point of contact with the allergen can determine the organs or tissues where mast cells and Th2 cells are activated. For example, inhaled antigens cause rhinitis or asthma and ingested antigens often cause vomiting and diarrhea. Although there is a propensity for local reactions to depend on the site of allergen entry, many allergens can become widely disseminated whether inhaled or ingested and can cause symptoms throughout the body regardless of the site of entry. Injected antigens, such as drugs, can rapidly cause systemic effects. The concentration of mast cells in various target organs also influences the severity of responses. Mast cells are particularly abundant in the skin and the mucosa of the respiratory and gastrointestinal tracts, and these tissues frequently suffer the most injury in immediate hypersensitivity reactions. The local mast cell phenotype may influence the characteristics of the immediate hypersensitivity reaction. For example, connective tissue mast cells produce abundant histamine and are responsible for wheal-and-flare reactions in the skin.

In the following section, we will discuss the major features of allergic diseases manifested in different tissues.

Systemic Anaphylaxis

Anaphylaxis is a systemic immediate hypersensitivity reaction characterized by edema in many tissues and a decrease in blood pressure secondary to vasodilation and vascular leak. These effects usually result from the systemic presence of antigen introduced by injection, an insect sting, or absorption across an epithelial surface such as gut mucosa. The allergens that most often cause anaphylaxis include penicillin family antibiotics and proteins in peanuts, tree nuts, fish, shellfish, milk, eggs, and bee venom, but there are many other drug, food, and environmental culprits. The allergen activates mast cells in many tissues, resulting in the release of mediators that gain access to vascular beds throughout the body. The decrease in vascular tone and leakage of plasma caused by mast cell mediators can lead to a significant decrease in blood pressure, or shock, called anaphylactic shock, which is often fatal. Mast cell mediators may impair breathing by causing laryngeal edema, bronchoconstriction and excess bronchial mucus production. There is often diarrhea as a result of intestinal hypermotility and outpouring of mucus in the gut and urticarial lesions (hives) in the skin. Anaphylaxis usually occurs within seconds to an hour of exposure to an allergen. In about 20% of patients a second recurrence of symptoms is seen without known reexposure to the allergen, up to 12 hours after the first episode. This is often called a late-phase anaphylactic reaction but should not be confused with the late-phase reaction to allergen discussed earlier. It is not known which mast cell mediators are the most important in anaphylactic shock. The mainstay of treatment is epinephrine injection, which can be lifesaving by reversing the bronchoconstrictive and vasodilatory effects of mast cell mediators.

Epinephrine also improves cardiac output, further aiding survival from threatened circulatory collapse. Antihistamines are often given to patients with anaphylaxis, but their effectiveness is not proved.

Asthma

Asthma includes a group of pulmonary diseases characterized by recurrent reversible airflow obstruction and bronchial smooth muscle cell hyperresponsiveness, most often caused by repeated immediate-type hypersensitivity and late-phase reactions (Fig. 20.9). Patients have paroxysms of bronchoconstriction and increased production of thick mucus, which lead to bronchial obstruction and respiratory difficulties. Asthma in adults frequently coexists with chronic obstructive pulmonary disease, and the combination of these diseases can cause severe irreversible airflow obstruction. Affected individuals may have considerable morbidity, and asthma can be fatal. Asthma affects approximately 25 million people in the United States, and the frequency of this disease has increased over the past 30 to 40 years. The prevalence rate is similar in other industrialized countries and higher than in lower-income areas of the world.

Approximately 70% of cases of asthma are associated with IgE-mediated reactions reflecting atopy. In the remaining 30% of patients, asthma may be triggered by nonimmune stimuli, such as drugs, cold, and exercise. Even among nonatopic asthmatics, the pathophysiologic process of airway constriction is similar, which suggests that alternative mechanisms of mast cell degranulation (e.g., by locally produced neurotransmitters) may underlie the disease.

The pathophysiologic sequence in atopic asthma is probably initiated by mast cell activation in response to allergen binding to IgE and by Th2 cells reacting to allergens (Fig. 20.10). The lipid mediators and cytokines produced by the mast cells and T cells lead to the recruitment of eosinophils, basophils, and more Th2 cells. The chronic inflammation in this disease may continue without mast cell activation. There is experimental evidence that other T cell subsets, including Th1 and Th17 cells and IL-9–secreting T cells, may contribute to the pathologic processes in established disease. Smooth muscle cell hypertrophy and hyperreactivity are thought to result from leukocyte-derived mediators and cytokines. Mast cells, basophils, and eosinophils all produce mediators that constrict airway smooth muscle. The most important of the bronchoconstricting mediators are cysteinyl leukotrienes, including LTC_4 and its metabolites. Increased mucus secretion results from the action of cytokines, mainly IL-13, on bronchial epithelial cells.

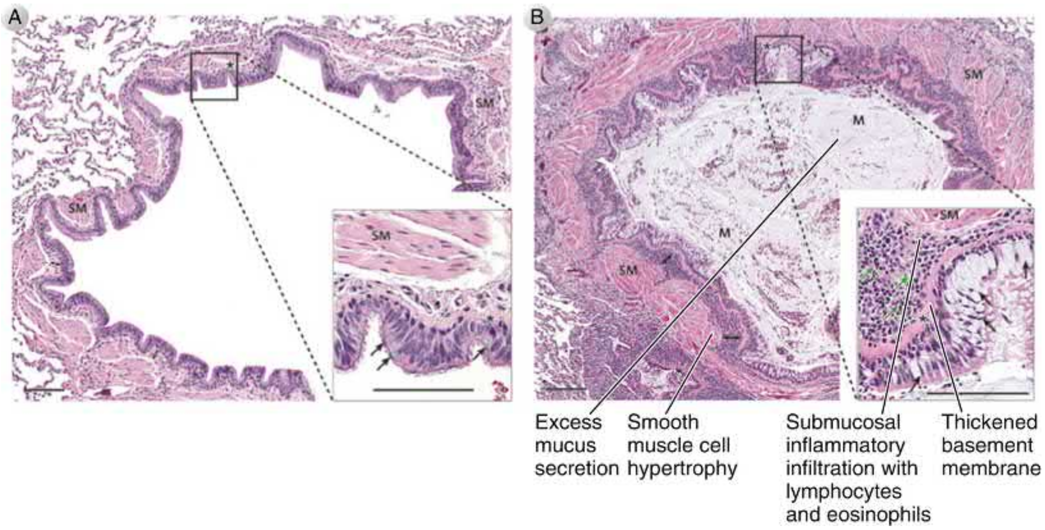

A
SM
SM
SM
SM
B
M
M
SM
SM
SM
Excess mucus secretion
Smooth muscle cell hypertrophy
Submucosal inflammatory infiltration with lymphocytes and eosinophils
Thickened basement membrane

FIGURE 20.9 Histopathologic features of bronchial asthma.Atopic bronchial asthma results from repeated immediate hypersensitivity reactions in the lungs with chronic late-phase reactions. A cross-section of a normal bronchus **(A)** and a cross-section of a bronchus from a patient with asthma **(B)** are shown. The diseased bronchus has excessive mucus *(M)* production, many submucosal inflammatory cells (including eosinophils), smooth muscle *(SM)* hypertrophy, and many more goblet cells than in the normal bronchus *(black arrows in insets).*

From Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445–454. Courtesy G. J. Berry, Stanford University, California.

Subsets of patients with severe asthma can be segregated based on high or low levels of biomarkers reflecting type 2 inflammation. These markers include sputum and blood eosinophil counts as well as type 2 cytokine gene expression signatures in bronchial biopsies. Although there is significant heterogeneity of clinical features even among patients with type 2 high and low disease phenotypes, this classification has proved useful to determine which patients are most likely to benefit from new cytokine-directed therapies for asthma, discussed next.

Current therapy for asthma has two major goals: prevention and reversal of inflammation, and relaxation of airway smooth muscle (see Fig. 20.10). Several classes of drugs are in current use to treat asthma, but antiinflammatory agents are now the primary mode of treatment. Inhaled corticosteroids block the production of inflammatory cytokines. Corticosteroids also may be given systemically, especially once an attack is under way, to reduce inflammation. Bronchial smooth muscle cell relaxation is achieved principally by drugs that elevate intracellular cyclic adenosine monophosphate (cAMP) levels in smooth muscle cells, which inhibits contraction. The major drugs used to elevate cAMP are activators of adenylate cyclase, including inhaled long-acting β_2-adrenergic agonists. Antagonists specific for LTC_4 receptor on airway smooth muscle cells are effective in reducing bronchoconstriction. A humanized monoclonal anti-IgE antibody is an approved therapy that effectively reduces serum IgE levels in patients. Several monoclonal antibody drugs specific for cytokines or cytokine receptors have been approved for treatment of asthma, including antibodies specific for IL-5, IL-5 receptor, IL-4RA (which is shared by both IL-4 and IL-13 receptors), and TSLP. These biologic drugs are mainly used in patients with severe type 2-high disease that is refractory to other treatments. Because histamine has little role in airway constriction, antihistamines (histamine-1 [H1] receptor antagonists) are not useful in the treatment of asthma. Indeed, because many antihistamines are also anticholinergics, these drugs may worsen airway obstruction by causing thickening of mucus secretions.

Immediate Hypersensitivity Reactions in the Upper Respiratory Tract, Gastrointestinal Tract, and Skin

Allergic rhinitis, also called hay fever, is perhaps the most prevalent allergic disease and is a consequence of immediate hypersensitivity reactions to common allergens such as plant pollen or house dust mites localized to the upper respiratory tract by inhalation. The pathologic and clinical manifestations include mucosal edema, leukocyte infiltration with abundant eosinophils, mucus secretion, coughing, sneezing, and difficulty breathing. Allergic conjunctivitis with itchy eyes is commonly associated with the rhinitis. Focal protrusions of the nasal mucosa, called nasal polyps, filled with edema fluid and eosinophils may develop in patients who have frequent repetitive bouts of allergic rhinitis. Antihistamines are commonly used to treat allergic rhinitis.

Food allergies are immediate hypersensitivity reactions to ingested foods that lead to the release of mediators from intestinal mucosal and submucosal mast cells of the gastrointestinal tract, including the oropharynx. The resulting clinical manifestations include pruritus, tissue edema, enhanced peristalsis, increased epithelial fluid secretion, and symptoms of oropharyngeal swelling, vomiting, and diarrhea. Rhinitis, urticaria, and mild bronchospasm are also often associated with allergic reactions to food, suggestive of systemic antigen exposure, and anaphylaxis may occasionally occur. Individuals may be sufficiently sensitive to these allergens that severe systemic reactions can occur in response to small accidental ingestions. Allergies to foods, including cow's milk, eggs, peanuts, tree nuts, shellfish, fish, soy, and wheat, are extremely common across the world.

Common allergic reactions in the skin include urticaria and atopic dermatitis. **Urticaria,** or hives, is an acute wheal-and-flare reaction induced by mast cell mediators and occurs in response to direct local contact with an allergen or after an allergen enters the circulation. Because the reaction that ensues is mediated largely by histamine, antihistamines can attenuate this response and are the mainstay of therapy. Urticaria may persist for several hours or days. Rare cases of chronic urticaria are due to IgG autoantibodies specific for FcεR1or the Fc portion of IgE.

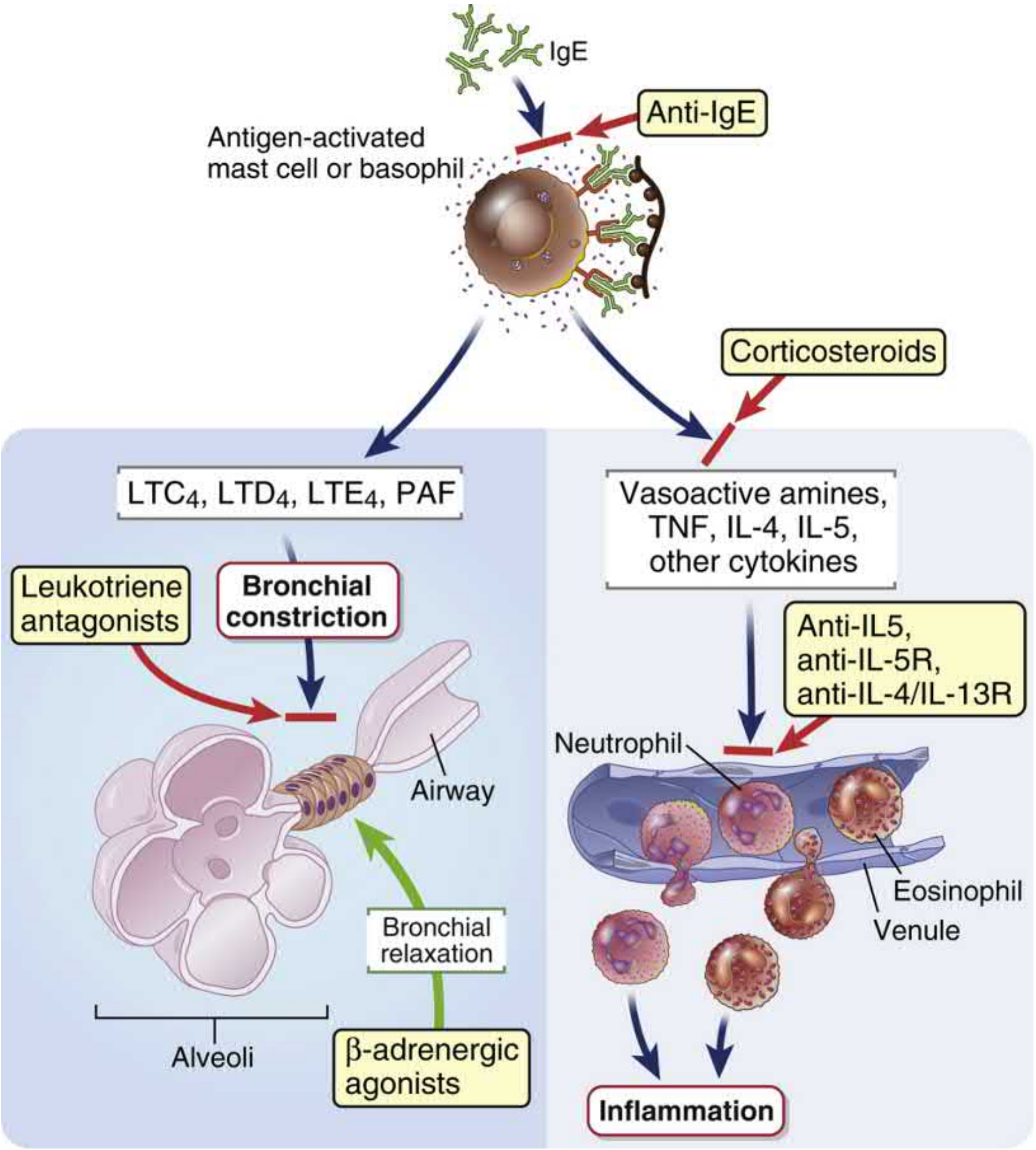

IgE
Anti-IgE
Antigen-activated mast cell or basophil
Corticosteroids
LTC4, LTD4, LTE4, PAF
Vasoactive amines, TNF, IL-4, IL-5, other cytokines
Leukotriene antagonists
Bronchial constriction
Anti-IL5, anti-IL-5R, anti-IL-4/IL-13R
Neutrophil
Airway
Eosinophil
Venule
Bronchial relaxation
Alveoli
β-adrenergic agonists
Inflammation

FIGURE 20.10 Mediators and treatment of asthma.Mast cell–derived leukotrienes and platelet-activating factor *(PAF)* are thought to be the major mediators of acute bronchoconstriction. Therapy is targeted at reducing mast cell activation with anti–immunoglobulin E *(IgE)* and mast cell degranulation with inhibitors such as cromolyn and at countering mediator actions on bronchial smooth muscle by leukotriene antagonists and bronchodilators such as inhaled β-adrenergic receptor agonists. Mast cell–derived

cytokines are thought to be the major mediators of sustained airway inflammation, which is an example of a late-phase reaction; corticosteroid therapy is used to inhibit cytokine synthesis, and antibodies are used block the actions of the cytokines. Cytokines are also produced by helper T cells *(not shown). IL,* Interleukin; *LTC* $_4$, leukotriene C_4; *PAF,* platelet-activating factor; *TNF,* tumor necrosis factor.

Atopic dermatitis (commonly called **eczema**) is characterized by acute flares of itchy red exudative papules and chronically dry scaly skin. It is part of the atopic triad (atopic dermatitis, allergic rhinitis, and asthma) discussed earlier, but it can also occur in isolation. It is a common skin disorder, sometimes associated with filaggrin mutations that result in defective skin barrier function. As a result, there is increased exposure to environmental antigens and activation of keratinocytes to secrete cytokines that promote type 2 immune responses. Patients with eczema go on to develop chronic late-phase reactions in the skin. As may be expected for a cytokine-mediated response, the late-phase inflammatory reaction is not inhibited by antihistamines but can be treated with corticosteroids, which inhibit cytokine synthesis. Anti–IL-4R antibody is approved for the treatment of atopic dermatitis and chronic urticaria.

Specific Immunotherapy (Desensitization) for Allergic Diseases

In addition to therapy aimed at the consequences of immediate hypersensitivity that we have discussed, clinical immunologists often try to reduce the onset of allergic reactions by altering the allergen-specific immune response in the patient. Several empirical immunotherapy protocols have been used, which induce multiple immunologic alterations that may account for the clinical benefit. In one approach, called **desensitization,** or specific allergen immunotherapy, small quantities of the allergen are repeatedly administered subcutaneously or sublingually. As a result of this treatment, specific IgE levels decrease and IgG titers often rise, perhaps further inhibiting IgE production by neutralizing the antigen and by antibody feedback (see Chapter 12). It is possible that desensitization may work by inducing specific T cell tolerance, by changing the predominant phenotype of antigen-specific T cells from Th2 to Th1, by inducing production of non-allergy isotypes of IgG specific for the allergen, or by inducing allergen-specific regulatory T cells; however, there is no clear evidence to support any of these hypotheses. The beneficial effects of desensitization may occur in a matter of hours, much earlier than changes in IgE levels. Although the precise mechanism is not known, this approach has been effective in preventing acute anaphylactic responses to protein antigens (e.g., insect venom) or vital drugs (e.g., penicillin). Many people with more common chronic atopic conditions, such as hay fever and asthma, also benefit from desensitization therapy, but the overall effectiveness for allergic disorders is more variable. It is now possible to identify the allergens that bind to IgE in each patient, using chip-based antibody-binding assays, and this may greatly facilitate the development of antigen-specific immunotherapy.

Epidemiologic and clinical trial data have shown that exposure of infants 4 to 11 months of age to peanut-containing foods reduces the risk of developing peanut allergy later in life. These results have led to reversal of standard clinical recommendations, from peanut avoidance to peanut exposure, for all children at risk for developing peanut allergy (e.g., children with a strong family history or severe eczema). In the trials, prevention of allergy by early-life exposure correlated with induction of non-allergenic IgG4 antibodies specific for the peanut allergens, but it is not known if this or other mechanisms are the basis of tolerance induction, and it is not known if this approach of early-life exposure will be effective for other food allergens. A preparation of powdered peanuts taken orally has been shown to reduce risks for severe allergic reactions to peanuts in children older than 4 years with known peanut allergy and is approved for use in children 4 to 17 years of age.

The Protective Roles of Immune Reactions Mediated by IgE and Mast Cells

Although IgE- and mast cell–mediated reactions are implicated mostly in immediate hypersensitivity, it is reasonable to assume that these responses have evolved because they provide protective functions. This assumption is supported by the correlation of some infections with elevated IgE levels and eosinophilia. Studies in mice that were deficient in IgE, Th2 cytokines, or mast cells have provided evidence that IgE- and mast cell–mediated responses are important for defense against certain types of infection.

IgE-initiated immune reactions may contribute to the eradication of various microbes, including helminthic parasites. Eosinophil-mediated killing of helminths is an effective defense against these organisms (see Chapter 10). Some evidence indicates that the activities of IL-4 and IL-13 in IgE production and IL-5 in eosinophil activation contribute to a coordinated defense against helminths. In addition, IgE-dependent mast cell activation in the gastrointestinal tract promotes the expulsion of parasites by increasing peristalsis and by an outpouring of mucus. Studies in mice have highlighted these important roles of IgE and mast cells. For example, mice treated with anti–IL-4 antibody and IL-4 knockout mice do not make IgE and appear to be more susceptible than normal animals to some helminthic infections. IL-5 knockout mice, which are unable to activate eosinophils, also show increased susceptibility to some helminths. Furthermore, genetically mast cell–deficient mice show increased susceptibility to infection by tick larvae, and immunity can be provided to these mice by adoptive transfer of specific IgE and mast cells (but not by either component alone). The larvae are eradicated by the late-phase reaction. Nonetheless, the role of type 2 responses in protecting humans from helminths is controversial, and human worm infections are frequently sustained for decades in the face of chronic type 2 responses.

Mast cells play an important protective role as part of the innate immune response to bacterial infections and venoms. Studies in mice have indicated that mast cells can be activated by IgE-independent mechanisms in the course of an acute bacterial infection and that the mediators they release are critical for clearing the infection. Mast cell–deficient mice are less capable of clearing and are more likely to die of acute bacterial infection of the peritoneum than are normal mice. The protective role of mast cells in this setting is mediated by TNF and depends on TNF-stimulated influx of neutrophils into the peritoneum, specifically the late-phase reaction. The mechanisms by which mast cells are activated during innate immune responses to bacterial infection include binding of pathogen-associated molecular patterns to TLRs on mast cells and complement activation by the alternative pathway, leading to the release of C5a, which directly triggers mast cell degranulation.

Mast cell–derived proteases have been shown to destroy some snake and insect venoms in mice, and venom-specific IgE confers protection from envenomation. This is an unusual form of immunity against a potentially lethal encounter with nonmicrobial organisms and their toxins.

Summary

- Immediate hypersensitivity is an immune reaction triggered by mast cell activation, usually by antigen binding to IgE (immunoglobulin E) attached to mast cells.
- The steps in the development of immediate hypersensitivity are exposure to an antigen (allergen) that stimulates type 2 responses, characterized by production of the cytokines interleukin-4 (IL-4), IL-5, and IL-13, IgE production, binding of the IgE to Fcε receptors on mast cells, cross-linking of the bound IgE by the allergen, activation of mast cells, and release of mediators.

- Individuals who are susceptible to immediate hypersensitivity reactions are called atopic and often have more IgE in the blood and more IgE-specific Fc receptors per mast cell than do nonatopic individuals. IgE synthesis by B cells is induced by exposure to antigen and IL-4 and IL-13 secreted by T follicular helper (Tfh) cells.
- Atopic diseases are characterized by repetitive bouts of type 2 inflammation involving various cell types, including Th2 cells, innate lymphoid cells (ILC2s), mast cells, basophils, and eosinophils.
- Mast cells are derived from bone marrow precursors that mature in tissues. They express high-affinity receptors for IgE (FcεRI) and contain cytoplasmic granules in which various inflammatory mediators are stored. Basophils are a type of circulating granulocyte that also express high-affinity Fcε receptors and contain granules with contents similar to those of mast cells.
- Eosinophils are a special class of granulocyte; they are recruited into inflammatory reactions by chemokines and IL-4 and are activated by IL-5. Eosinophils are effector cells that are involved in killing parasites. In allergic reactions, eosinophils contribute to tissue injury.
- On binding of antigen to IgE on the surface of mast cells or basophils, the high-affinity Fcε receptors become cross-linked and activate intracellular signaling pathways that lead to granule exocytosis, releasing histamine and other vasoactive amines and proteases. In response to allergen, mast cells and basophils are also activated to synthesize and secrete lipid mediators, such as prostaglandins, leukotrienes, and platelet-activating factor, and cytokines, such as tumor necrosis factor, IL-4, IL-13, and IL-5.
- Vasoactive amines and lipid mediators cause the rapid vascular and smooth muscle reactions of immediate hypersensitivity, such as vasodilation, vascular leakage and edema, bronchoconstriction, and gut hypermotility. Cytokines released by mast cells and Th2 cells mediate the

late-phase reaction, which is an inflammatory reaction involving neutrophil and eosinophil infiltration.

- Susceptibly to allergic diseases is inherited, and allelic variations of several genes have been associated with allergic asthma. Genetic susceptibly interacts with environmental factors to result in atopy.
- Various organs show distinct forms of immediate hypersensitivity involving different mediators and target cell types. The most severe and often fatal form is a systemic reaction called anaphylactic shock, characterized by diffuse edema with reduced blood volume, and airway obstruction. Asthma is a chronic airway disease with bronchial inflammation and episodes of reversible bronchial constriction. Most cases of asthma are a manifestation of repetitive immediate hypersensitivity reactions in the lung. Allergic rhinitis (hay fever) is the most common allergic disease of the upper respiratory tract. Food allergens can cause diarrhea and vomiting. In the skin, immediate hypersensitivity is manifested as wheal-and-flare (hives) and late-phase reactions and may lead to chronic atopic dermatitis (eczema).
- Drug therapy is aimed at inhibiting mast cell mediator production and at blocking or counteracting the effects of released mediators on target organs. Monoclonal antibodies against cytokines, cytokine receptors, and IgE are approved for some allergic diseases, including asthma. Desensitization immunotherapy involves controlled exposure to specific allergens with a goal of preventing or reducing Th2 cell responses and the production of IgE specific for those allergens.
- Immediate hypersensitivity reactions provide protection against helminthic infections by promoting eosinophil-mediated cytotoxicity and gut peristalsis. Mast cells may also play a role in innate immune responses to bacterial infections.

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*Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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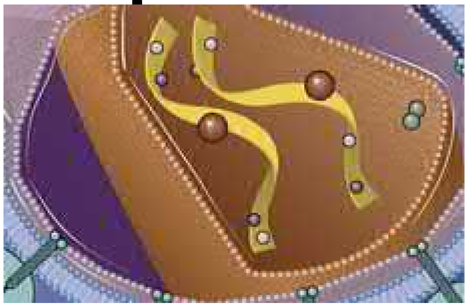
Chapter 21: Primary and Acquired Immunodeficiencies



Overview of Immunodeficiency Diseases,

Primary (Congenital) Immunodeficiencies,
  Defects in Innate Immunity,
  Severe Combined Immunodeficiency,
  Antibody Deficiencies: Defects in B Cell Development and Activation,
  Defects in T Lymphocyte Activation and Function,
  Multisystem Disorders With Immunodeficiency,
  Therapeutic Approaches for Primary Immunodeficiencies,

Secondary (Acquired) Immunodeficiencies,

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome,
  An Overview of HIV Virology,
  Pathogenesis of HIV Infection and AIDS,

  - Clinical Features of HIV Disease,
  - Immune Responses to HIV,
  - Elite Controllers and Long-Term Nonprogressors: A Possible Role for Host Genes,
  - Treatment and Prevention of AIDS and Vaccine Development,
- Summary,

Integrity of the immune system is essential for defense against infectious organisms and their toxic products and therefore for the survival of all individuals. Defects in one or more components of the immune system can lead to serious and often fatal disorders, which are collectively called **immunodeficiency diseases.** These diseases are broadly classified into two groups. The **primary immunodeficiencies** are genetic defects that result in an increased susceptibility to infection that is frequently manifested in infancy and early childhood but is sometimes first clinically detected later in life. A large number of individuals may inherit mutant genes that compromise the function of the immune system. However, a smaller fraction inherit deleterious mutations that on their own have large enough effects on the development or function of the immune system to be categorized as primary immunodeficiency disorders. Estimates of how prevalent primary immunodeficiencies are in the United States range from 1 in 1200 to 1 in 10,000, although only a small proportion of these are affected severely enough for development of life-threatening complications. **Secondary,** or **acquired, immunodeficiencies** are not inherited diseases but develop as a consequence of malnutrition, disseminated cancer, treatment with immunosuppressive drugs, or infection of cells of the immune system, most notably with the human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS). This chapter describes the major types of congenital and acquired immunodeficiencies, with an emphasis on their pathogenesis and the components of the immune system that are involved in these disorders.

Overview of Immunodeficiency Diseases

Before beginning our discussion of individual diseases, it is important to summarize some general features of immunodeficiencies.

The principal consequence of immunodeficiency is increased susceptibility to infection. The nature of the infection in a particular patient depends largely on the component of the immune system that is defective (Table 21.1). Deficient humoral immunity usually results in infection by encapsulated, pus-forming bacteria and some viruses, whereas defects in cell-mediated immunity lead to infection by viruses and other intracellular microbes or the reactivation of latent infections. Combined deficiencies in both humoral and cell-mediated immunity make patients susceptible to infection by all classes of microorganisms. Immunodeficient patients often present with infections by microbes that are commonly encountered but effectively eliminated by healthy persons; such infections are said to be **opportunistic.** Defects in innate immunity can result in infections by different categories of microbes, depending on the pathway or cell type affected. In addition to the clinically well-defined immunodeficiency diseases, there is growing evidence that adults with recurrent or severe infections may harbor mutations in genes that regulate immune function.

Table 21.1

Features of Immunodeficiencies Affecting T or B Lymphocytes

Feature	B Cell Deficiency	T Cell Deficiency
Susceptibility to infection	Pyogenic bacteria (otitis, pneumonia, meningitis, osteomyelitis), enteric bacteria and viruses, some parasites	*Pneumocystis jiroveci*, many viruses, atypical mycobacteria, fungi
Serum immunoglobulin levels	Reduced	Normal or reduced
DTH reactions to common antigens	Normal	Reduced
Morphology of lymphoid tissues	Absent or reduced follicles and germinal centers (B cell zones)	Usually normal follicles, may be reduced parafollicular cortical regions (T cell zones)

DTH, Delayed-type hypersensitivity.

Patients with immunodeficiencies are also susceptible to certain types of cancer. Many of these cancers appear to be caused by oncogenic viruses, such as the Epstein-Barr virus (EBV) and human papillomaviruses (HPVs), and hence reflect defective antiviral immunity, but the incidence of lymphoma and cancers of the skin, stomach, and other organs with no known association with viral infections is also increased.

In this chapter, we first describe primary (congenital) immunodeficiencies, including defects in components of the innate immune system and defects in the humoral and cell-mediated arms of the adaptive immune system. We conclude with a discussion of secondary (acquired) immunodeficiencies, with an emphasis on AIDS.

Primary (Congenital) Immunodeficiencies

Primary immunodeficiencies are monogenic diseases caused by germline mutations in genes that regulate the development or function of the immune system. Although the earliest primary immunodeficiency disorders in which precise genetic defects were identified were X-linked recessive diseases, the majority of primary immunodeficiencies exhibit an autosomal recessive inheritance. Autosomal recessive alleles are often seen in consanguineous families when the same mutation is inherited from both parents. In other cases, especially in offspring of nonconsanguineous marriages, one defective allele of a specific gene is inherited from one parent and a different defective mutation in the same gene is inherited from the other parent; individuals with this kind of autosomal recessive inheritance pattern are referred to as compound heterozygotes. Sometimes a compound heterozygote may include one or both partially defective or hypomorphic alleles.

Some primary immunodeficiencies are associated with autosomal dominant inheritance. These include gain-of-function mutations, such as activating mutations in *PIK3CD* (encoding a hyperactive form of PI3 kinase δ). Other dominantly inherited mutations include loss-of-function mutations in one allele of a gene that leads to impaired function but not complete loss of a specific protein. These may cause disease because of haploinsufficiency, seen with mutations in *CTLA4*, or because of a dominant negative effect whereby one defective gene product impairs the activity of the protein product of the normal allele. Occasionally, the causative mutation arises de novo in the patient and is not present in either parent. These noninherited germline mutations typically occur in a germ cell of one of the parents or in the fertilized egg.

The expression and clinical presentation of diseases caused by the same mutation may be variable. Multiple factors may contribute to the phenotypic variability, including the coinheritance of modifier genes, environmental factors, and epigenetic modifications of genes that vary from one individual to another. However, in most cases, different phenotypic manifestations of the same mutation remain unexplained.

Primary immunodeficiency diseases generally come to light because of a clinical history of repeated infections. Some diagnoses are quite easily made by measurement of serum immunoglobulin (Ig) levels, flow cytometry of immune cells, or assessment of neutrophil function in vitro. However, more detailed investigations are often necessary to obtain an accurate diagnosis. Primary T cell immunodeficiencies are diagnosed by reduced numbers of peripheral blood T cells, low proliferative responses of blood lymphocytes to polyclonal T cell activators such as phytohemagglutinin, and deficient cutaneous delayed-type hypersensitivity (DTH) reactions to ubiquitous microbial antigens, such as *Candida* antigens. In the United States, it is now required that newborns be screened using an assay for T cell receptor excision circles (TRECs) in blood cells, looking for DNA that is deleted during T cell receptor (TCR) gene rearrangement in developing T cells. The failure to detect these DNA circles indicates an absence of T cell development. This assay is used to diagnose severe combined immunodeficiency, discussed later, immediately after birth and allows for timely correction of the defect by hematopoietic stem cell transplantation. Early diagnosis may also make gene therapy feasible before significant disease has developed.

Immunodeficiency may result from defects in lymphocyte development or activation or from defects in the effector mechanisms of innate and adaptive immunity. Immunodeficiency diseases are clinically and pathologically heterogeneous, in part because different diseases involve different components of the immune system. Abnormalities in lymphocyte development may be caused by mutations in genes encoding receptors and other molecules involved in signaling, including enzymes, adaptors, transport proteins, and transcription factors. These inherited defects, and the corresponding targeted disruptions in mice, have been instructive in elucidating mechanisms of lymphocyte development and function (see Chapter 8).

The availability of new rapid and efficient DNA sequencing technology has greatly increased our ability to identify specific genes that, when mutated, confer susceptibility to pathogens. One of the surprising lessons that is emerging from the analysis of immunodeficiencies caused by single-gene mutations is that many of them make individuals susceptible to a restricted set of infections. This observation suggests that in humans, distinct protective mechanisms are often critical for defense against specific pathogens, so that defects in any one mechanism make individuals susceptible to only some infections.

Paradoxically, certain immunodeficiencies are associated with an increased incidence of autoimmunity. Autoimmunity is generally seen in immunodeficiencies in which there is an incomplete loss of an immune population or function because of a hypomorphic mutation, possibly resulting in attenuation of some regulatory mechanism. It is also possible that the persistent infections associated with immunodeficiencies cause innate immune activation and tissue injury and promote the activation of autoreactive lymphocytes.

In the following sections, we will describe immunodeficiencies caused by inherited mutations in genes encoding components of the innate immune system or in genes required for lymphocyte development and activation. Many of the known mutations are listed in the tables, and only the most common and those that illustrate important principles are described in the text. We conclude with a brief discussion of therapeutic strategies for these diseases.

Defects in Innate Immunity

Innate immunity constitutes the first line of defense against infectious organisms; hence, congenital disorders of components of innate immunity often result in recurrent infections. The mechanisms of innate immunity were described in Chapter 4. In this section of the chapter, we will discuss some examples of congenital phagocyte disorders, natural killer (NK) cell deficiencies, and genetic defects in Toll-like receptor (TLR) signaling and in the interleukin-12 (IL-12)/interferon-γ (IFN-γ) pathway (Table 21.2). Phagocyte defects generally result in infections of the skin and respiratory tract with bacteria or fungi, the latter predominantly involving *Aspergillus* and *Candida* species. Deep-seated abscesses and oral stomatitis are also common. Defects in TLR signaling and in type 1 IFN signaling may contribute to recurrent pyogenic infections and to severe viral infections; defects in IL-12 and the IFN-γ pathway increase susceptibility to intracellular pathogens, particularly mycobacterial infections. Complement deficiencies are described in Chapter 13.

Defective Microbicidal Activity of Phagocytes: Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is caused by mutations in components of the phagocyte oxidase (PHOX) enzyme complex. It is a rare disease, estimated to affect approximately 1 in 200,000 individuals in the United States. Approximately two-thirds of cases show an X-linked recessive pattern of inheritance, and the remainder are autosomal recessive. In the X-linked form of the disease, there is a mutation in the gene encoding the 91-kD α subunit of cytochrome *b*558, an integral membrane protein also known as PHOX-91. Many different mutations in this X-linked gene have been identified, and these mutations result in defective production of superoxide anion, one of several reactive oxygen species that constitute a major microbicidal mechanism of phagocytes, especially neutrophils (see Chapter 4). Defective production of reactive oxygen species results in a failure to kill phagocytosed microbes. Mutations in other components of the PHOX complex cause autosomal recessive forms of CGD.

CGD is characterized by recurrent infections with fungi and bacteria, such as *Staphylococcus,* usually from early childhood. Invasive infection with the fungus *Aspergillus* is the leading cause of death. Many of the organisms that are particularly troublesome in patients with CGD produce catalase, which destroys the microbicidal hydrogen peroxide that may be produced by host cells from the residual reactive oxygen radical, superoxide. Because the infections are not controlled by neutrophils, they stimulate chronic cell-mediated immune responses, resulting in T cell–mediated macrophage activation and the formation of granulomas composed of activated macrophages, which try to eliminate the microbes. This histologic appearance is the basis for the name of the disorder. The disease was often fatal in the past, even with aggressive antibiotic therapy, but the prognosis has improved significantly now because of earlier recognition and better control of infections.

The cytokine IFN-γ enhances transcription of the gene encoding PHOX-91 and also stimulates other components of the PHOX enzyme complex. Therefore, IFN-γ stimulates the production of superoxide by CGD neutrophils, especially in cases in which the coding portion of the *PHOX-91* gene is intact but its transcription is reduced. If neutrophil superoxide production is restored to at least 10% of normal levels, resistance to infection is greatly improved. In the United States, IFN-γ therapy is now used in some medical centers for the treatment of X-linked CGD.

Leukocyte Adhesion Deficiencies

The leukocyte adhesion deficiencies are a group of autosomal recessive disorders caused by defects in leukocyte and endothelial adhesion molecules. These diseases are characterized by a failure of leukocyte, particularly neutrophil, recruitment to sites of infection, resulting in severe periodontitis and other recurrent bacterial infections starting early in life, and an inability to make pus.

Different types of leukocyte adhesion deficiencies are caused by mutations in different genes.

Table 21.2

Congenital Disorders of Innate Immunity

Disease	Functional Deficiencies	Mechanism of Defect
Chronic granulomatous disease	Defective production of reactive oxygen species by phagocytes; recurrent bacterial and fungal infections	Mutation in genes encoding proteins of the phagocyte oxidase complex; *PHOX91* (cytochrome *b*558 α subunit) is mutated in the X-linked form
Leukocyte adhesion deficiency type 1	Defective leukocyte adhesion to endothelial cells and migration into tissues linked to decreased or absent expression of β_2 integrins; recurrent bacterial and fungal infections	Mutations in gene encoding the β chain (CD18) of β_2 integrins

Disease	Functional Deficiencies	Mechanism of Defect
Leukocyte adhesion deficiency type 2	Defective leukocyte rolling and migration into tissues linked to decreased or absent expression of leukocyte ligands for endothelial E- and P-selectins, causing failure of leukocyte migration into tissues; recurrent bacterial and fungal infections	Mutations in gene encoding GDP-fucose transporter-1, required for transport of fucose into the Golgi and its incorporation into sialyl Lewis X, the ligand for selectins
Leukocyte adhesion deficiency type 3	Defective leukocyte adhesion and migration into tissues linked to defective chemokine-stimulated integrin activation	Mutations in gene encoding KINDLIN-3, a cytoskeletal protein linked to integrin activation

Disease	Functional Deficiencies	Mechanism of Defect
Chédiak-Higashi syndrome	Defective vesicle fusion and lysosomal function in neutrophils, macrophages, dendritic cells, NK cells, cytotoxic T cells, and many other cell types; recurrent infections by pyogenic bacteria	Mutation in *LYST* leading to defect in secretory granule exocytosis and lysosomal function
NK cell deficiencies	Reduced or absent NK cells	Mutations in the gene encoding the GATA2 transcription factor and in the gene encoding the MCM4 DNA helicase
TLR signaling defects	Recurrent infections caused by defects in TLR and CD40 signaling and defective type I interferon production	Mutations in *TLR3, TRIF, TBK1, NEMO, UNC93B, MyD88, IκBα,* and *IRAK4* compromise NF-κB activation downstream of TLR

Disease	Functional Deficiencies	Mechanism of Defect
Mendelian susceptibility to mycobacterial diseases	Severe disease caused by nontuberculous environmental mycobacteria, BCG, *Salmonella*, and other intracellular bacteria	Mutations in *IL-12p40, IL-12RB, IFNGR1, IFNGR2, STAT1, NEMO*, and *ISG15*

BCG, Bacillus Calmette-Guérin; *GDP,* guanosine diphosphate; *IRAK-4,* IL-1 receptor–associated kinase 4; *LYST,* lysosomal trafficking protein; *NEMO,* NF-κB essential modulator; *NK cells,* natural killer cells; *TLR,* Toll-like receptor.

- ***Leukocyte adhesion deficiency type 1 (LAD-1)*** is a rare autosomal recessive disorder characterized by recurrent bacterial and fungal infections and impaired wound healing. Umbilical cord separation (which normally occurs as a consequence of inflammation and neutrophil infiltration) is delayed, and leukocytosis is common. In these patients, most adhesion-dependent functions of leukocytes are defective, including adherence to endothelium, neutrophil aggregation and chemotaxis, phagocytosis, and cytotoxicity mediated by neutrophils, NK cells, and T lymphocytes. The molecular basis of the defect is absent or reduced expression of the β_2 integrins (heterodimers of CD18 and the CD11 family of glycoproteins) as a result of mutations in the *CD18* gene. The β_2 integrins include leukocyte function–associated antigen 1 (LFA-1 or CD11aCD18), MAC-1 (CD11bCD18), and p150,95 (CD11cCD18). These proteins participate in the adhesion of leukocytes to other cells, notably endothelial cells, and the binding of T lymphocytes to antigen-presenting cells (APCs) (see Chapter 3).

- ***Leukocyte adhesion deficiency type 2 (LAD-2)*** is another rare disorder in which, as in LAD-1, children present with recurrent infections and leukocytosis, but they also exhibit severe developmental abnormalities. LAD-2 results from an absence of sialyl Lewis X, the tetrasaccharide carbohydrate ligand on neutrophils and other leukocytes that is required for binding of these cells to E-selectin and P-selectin on cytokine-activated endothelium (see Chapter 3). This defect is caused by a mutation in a guanosine diphosphate (GDP)-fucose transporter responsible for the transport of fucose into the Golgi, resulting in an inability to synthesize sialyl Lewis X. The absence of sialyl Lewis X results in defective attachment of leukocytes to endothelium, the absence of leukocyte rolling, and therefore the defective recruitment of leukocytes to sites of infection. This abnormality in fucosylation seen in LAD-2 also contributes to a Bombay blood group phenotype, which is a lack of the fucosylated H glycan that forms the core of the A, B, and O blood group antigens. Because LAD-2 has many nonimmunological manifestations related to defective fucose metabolism, it is also called congenital disorder of glycosylation (CDG).
- ***Leukocyte adhesion deficiency type 3 (LAD-3)*** patients present clinically with repeated bacterial infections and delayed umbilical cord separation as in LAD-1 but also have a life-threatening bleeding disorder that requires blood transfusions because of defective platelet aggregation, even though blood platelet counts are normal. This deficiency is caused by a defect in the inside-out signaling pathway that mediates chemokine-induced integrin activation that is required for leukocytes to bind firmly to endothelium (see Chapter 3) and for platelets to aggregate. In a subset of patients, it is caused by mutations in the gene encoding KINDLIN-3, a protein that binds to the cytoplasmic tail of some integrins and is involved in signaling.

Defects in NK Cells and Phagocytes

Rare patients lack NK cells because of autosomal dominant mutations in the gene encoding the GATA2 transcription factor. The loss of GATA2 activity results in diminished precursor populations in the bone marrow and a resulting loss of NK cells, as well as decreases in monocytes, dendritic cells (DCs), and B cells. Autosomal recessive mutations in *MCM4* (minichromosome maintenance complex component 4), a DNA helicase, also result in the loss of NK cells, accompanied by adrenal insufficiency and growth retardation. Autosomal recessive mutations affecting CD16 (FcγRIIIA), an Fc receptor that mediates antibody-dependent cell-mediated cytotoxicity (ADCC), result in a loss of NK cell function that goes beyond the loss of ADCC activity. Why CD16 is required broadly for NK cell function is unclear. Patients present with severe infections with viruses mainly of the herpesvirus and papillomavirus families.

Chédiak-Higashi syndrome is a rare autosomal recessive disorder characterized by recurrent infections by pyogenic bacteria, partial oculocutaneous albinism, and infiltration of various organs by nonneoplastic lymphocytes. The neutrophils, monocytes, and lymphocytes of these patients contain giant lysosomes. This disease is caused by mutations in the gene encoding the protein LYST, which regulates intracellular trafficking of lysosomes. The mutations result in defective phagosome-lysosome fusion in neutrophils and macrophages (causing reduced resistance to infection), defective melanosome formation in melanocytes (causing albinism), and lysosomal abnormalities in cells of the nervous system (causing nerve defects) and platelets (leading to bleeding disorders). Giant lysosomes form in neutrophils during the maturation of these cells from myeloid precursors. Some of these neutrophil precursors die prematurely, resulting in moderate leukopenia. Surviving neutrophils may contain reduced levels of the lysosomal enzymes that normally function in microbial killing. These cells are also defective in chemotaxis and phagocytosis, further contributing to their deficient microbicidal activity. NK cell function in these patients is impaired, probably because of an abnormality in the cytoplasmic granules that store proteins mediating cytotoxicity. The severity of the defect in cytotoxic T lymphocyte (CTL) function is variable among patients. A mouse strain called the beige mouse carries a mutation in the mouse homolog of the gene encoding LYST, and it is characterized by deficient NK cell function and giant lysosomes in leukocytes.

Inherited Defects in TLR Pathways, NF-κB Signaling, and Type 1 IFNs

Inherited defects in TLR-induced responses are rare and tend to cause fairly circumscribed clinical phenotypes. TLR3 mutations result in herpes simplex encephalitis. Almost all viruses, including DNA viruses such as the herpes virus, generate double-stranded RNA (dsRNA) transcripts, and these are recognized by TLR3 (see Chapter 4). The major signaling pathway downstream of most TLRs and of the IL-1 receptor (IL-1R) involves the MyD88 adaptor and the IRAK-4 and IRAK-1 kinases (see Chapter 4), and this pathway results in the NF-κB (nuclear factor-κB)-dependent induction of proinflammatory cytokines. Individuals with mutations in *MyD88* and *IRAK4* have severe invasive bacterial infections early in life, especially pneumococcal pneumonia. Later in life, infections tend to be less severe. TLR3 signaling uses the TRIF adaptor protein, instead of MyD88, and TBK1, a serine-threonine kinase that functions downstream of TRIF to activate IRF3 and NF-κB by the noncanonical pathway. Autosomal recessive mutations in *TRIF* and autosomal dominant mutations affecting the TRAF3 E3 ligase also result in susceptibility to herpes simplex encephalitis. A similar phenotype is observed with autosomal dominant mutations in the gene encoding TBK1. TLR3, 7, 8, and 9 recognize nucleic acids, are located in endosomes, and require a protein called UNC93B (Uncoordinated 93B) for their function. UNC93B is an endoplasmic reticulum membrane protein that interacts with endosomal TLRs when they are synthesized in the endoplasmic reticulum and helps deliver these TLRs to the endosomes. The UNC93B protein is also critical for signaling by the nucleic acid–specific TLRs. Homozygous mutations in *UNC93B* result in reduced type 1 IFN generation and increased susceptibility to herpes simplex encephalitis.

Signaling downstream of the endosomal TLRs results in the synthesis and secretion of type 1 IFNs, which bind to type 1 IFN receptors and activate the STAT1 (signal transducer and activator of transcription 1) transcription factor. In some patients, loss-of-function *STAT1* mutations are linked to severe viral infections, notably herpes simplex encephalitis. The finding that mutations in *TLR3* itself or in genes that affect TLR3 localization and signaling all result in susceptibility to herpes simplex encephalitis indicates that type 1 IFN production downstream of TLR3 activation is crucial in defense against this infection in the central nervous system (CNS). Interestingly, patients with severe COVID-19 (but not with less severe disease) frequently have mutations in TLR3 and downstream genes linked to type I interferon production.

Some immune deficiencies are caused by defects that specifically affect NF-κB activation. Point mutations in *IKKγ* (inhibitor of κB kinase γ), also known as *NEMO* (NF-κB essential modulator), which encodes a component of the IκB kinase complex that is required for NF-κB activation, contribute to the X-linked recessive disease known as anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID). In this disorder, differentiation of ectoderm-derived structures is abnormal, and immune function is impaired in a number of ways. Responses to TLR signals as well as CD40 signals are compromised. These patients suffer from infections with encapsulated pyogenic bacteria as well as with intracellular bacterial pathogens, including mycobacteria, viruses, and fungi such as *Pneumocystis jiroveci* (see also discussion later in the section on hyper-IgM syndromes).

Defects in the IL-12/IFN-γ Pathway

IL-12 is secreted by DCs and macrophages, and IL-12 receptor (IL-12R) signaling stimulates the synthesis of IFN-γ by helper T cells, cytotoxic T cells, and NK cells (see Chapters 4 and 10). Mutations in the genes encoding IL-12p40, the IL-12Rβ1 chain, and both chains of the IFN-γ receptor, as well as some mutations in *STAT1* and *IKKγ/NEMO,* result in susceptibility to environmental *Mycobacterium* species (often called atypical mycobacteria), such as *Mycobacterium avium, Mycobacterium kansasii,* and *Mycobacterium fortuitum.* The term Mendelian susceptibility to mycobacterial disease (MSMD) is used for these disorders, in which patients are predisposed to severe disease caused by weakly virulent mycobacteria that do not cause disease in healthy individuals, as well as other intracellular pathogens, including *Salmonella* and various other bacterial, fungal, and viral species.

Defects in Splenic Development

Splenic development may fail because of an autosomal dominant (and sometimes sporadic) condition called isolated congenital asplenia. In these patients, heterozygous missense mutations have been found in *NBX2.5,* which encodes a transcription factor. Asplenia also may be caused by mutations in genes controlling left-right laterality, which also affects other organs. Congenitally asplenic patients frequently suffer from severe infections with encapsulated bacteria, especially *Streptococcus pneumoniae*.

Severe Combined Immunodeficiency

Immunodeficiencies that affect both humoral and cell-mediated immunity are called severe combined immunodeficiency (SCID) (Table 21.3) **.** SCID usually results from impaired T lymphocyte development, resulting in defective cell-mediated immunity, with or without defects in B cell maturation. When there is no block in B cell development, the defect in humoral immunity is due to the absence of T cell help.

SCID patients suffer from severe infections that may be life-threatening, including pneumonia, meningitis, and bacteremia. Among the most dangerous organisms in the context of SCID is an intracellular fungus called *P. jiroveci,* which can cause a severe pneumonia in SCID patients. Many viruses cause serious disease in patients with SCID. Chickenpox (varicella) infection is usually limited to the skin and mucous membranes in immunologically normal children and typically resolves in days, but in patients with SCID, it can progress to involve the lungs, liver, and brain. Cytomegalovirus (CMV), which is present as a latent infection in most people, may be easily transmitted, often from mothers of SCID patients to their offspring, and cause fatal pneumonia in newborns. Children with SCID commonly develop gastrointestinal infections caused most often by rotavirus, CMV, or the protozoa *Cryptosporidium* and *Giardia lamblia,* leading to persistent diarrhea and malabsorption.

Children with SCID may also develop infections caused by live attenuated vaccines, which are not harmful in children who have normal immunity. Vaccines for chickenpox, measles, mumps, rubella, and rotavirus are live virus vaccines, and children with SCID can develop serious infections from these vaccines.

Some SCID patients develop a chronic skin rash that is often mistaken for infection. The rash is actually caused by a graft-versus-host reaction in which maternal T cells enter the fetus but are not rejected (because the fetus lacks a competent immune system) and react against paternal allogeneic antigens in the baby's tissues.

Mutations in genes involved in different steps in lymphocyte development may cause SCID (Fig. 21.1) **.** The process of T and B lymphocyte maturation from hematopoietic stem cells to functionally competent mature lymphocytes involves proliferation of lymphocyte progenitors, rearrangement of antigen receptor genes, and selection of cells with useful specificities (see Chapter 8). Defects in many of these steps have been described in different forms of SCID, including defects in thymus development, purine metabolism, and cytokine signaling. Approximately 50% of SCIDs are autosomal recessive; the rest are X-linked.

X-Linked SCID

X-linked SCID is caused by mutations in the gene encoding the common γ (γc) chain shared by the receptors for many cytokines,

including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (see Chapter 7) **.** X-linked SCID is characterized by impaired maturation of T cells and NK cells and greatly reduced numbers of mature T cells and NK cells, but the number of B cells is usually normal or increased. This disease is a result of the inability of the lymphopoietic cytokine IL-7, whose receptor uses the γc chain for signaling, to stimulate the growth of immature thymocytes. The humoral immunodeficiency in this disease is due to a lack of T cell help for antibody production. The receptor for IL-15, which is required for NK cell development, also uses the γc signaling chain, and the failure of IL-15 signaling accounts for the deficiency of NK cells.

Heterozygous females are usually phenotypically normal carriers, whereas males who inherit the abnormal X chromosome manifest the disease. Because developing cells in females randomly inactivate one of the two X chromosomes, the normal allele encoding a functional γc protein will not be expressed in half the lymphocyte precursors in a female carrier. These cells will fail to mature, and, consequently, all the mature lymphocytes in a female carrier will have inactivated the same X chromosome (carrying the mutant allele). In contrast, half of all nonlymphoid cells will have inactivated one X chromosome and half the other. A comparison of X chromosome inactivation in lymphocytes versus nonlymphoid cells is sometimes used to identify carriers of the mutant allele. The nonrandom use of X chromosomes in mature lymphocytes is also characteristic of female carriers of other mutated X-linked genes that affect lymphocyte development, as discussed later.

Table 21.3

Disease	Functional Deficiencies	Mechanism of Defect
Defective Thymic Development		
Defective pre-TCR checkpoint	Decreased T cells; normal or reduced B cells; reduced serum Ig	Mutations in *CD45, CD3D, CD3E, ORAI1* (CRAC channel component), *STIM1*
DiGeorge syndrome	Decreased T cells; normal B cells; normal or reduced serum Ig	22q11 deletion; T-box 1 (*TBX1*) transcription factor mutations
FOXN1 deficiency	Thymic aplasia with defective T cell development	Recessive mutation in *FOXN1*
TCR α chain deficiency	No αβ T cells; γδ T cells normal; recurrent infections and autoimmunity	Autosomal recessive deletion in C region of *TCR* α chain
Defective T cell thymic egress and defective T cell signaling	Marked reduction in all peripheral T cells	Mutations in *RHOH* and *MST1*
Selective loss of CD4^{+} T cells and defective T cell signaling	Decreased CD4^{+} T cells	Mutations in *LCK* and *UNC119*
Bare lymphocyte syndrome	Defective MHC class II expression and deficiency in CD4^{+} T cells; defective cell-mediated immunity and T-dependent humoral immune responses	Defects in transcription factors regulating MHC class II gene expression, including *CIITA, RFXANK, RFX5,* and *RFXAP*
MHC class I deficiency	Decreased MHC class I levels; reduced CD8^{+} T cells	Mutations in *TAP1, TAP2,* and *TAPASIN*
Reticular dysgenesis	Decreased T cells, B cells, and myeloid cells	Mutation in *AK2*
Defects in Nucleotide Salvage Pathways		
ADA deficiency	Progressive decrease in T cells, B cells, and NK cells; reduced serum Ig	Mutations in the *ADA* gene, leading to accumulation of toxic metabolites in lymphocytes
PNP deficiency	Progressive decrease in T cells, B cells, and NK cells; reduced serum Ig	Mutations in the *PNP* gene, leading to accumulation of toxic metabolites in lymphocytes
Defects in Cytokine Signaling		
X-linked SCID	Marked decrease in T cells; normal or increased B cells; reduced serum Ig	Cytokine receptor common γ chain mutations; defective T cell development because of the absence of IL-7–derived signals
Autosomal recessive SCID	Marked decrease in T cells; normal or increased B cells; reduced serum Ig	Mutations in *IL7RA, JAK3*
Defects in V(D)J Recombination		
RAG1 or RAG2 deficiency[a]	Decreased T cells and B cells; reduced serum Ig; absence or deficiency of T and B cells	Cleavage defect during V(D)J recombination; mutations in *RAG1* or *RAG2*
Double-stranded DNA break repair defects	Decreased T and B cells; reduced serum Ig; absence or deficiency of T cells and B cells	Failure to resolve hairpins during V(D)J recombination; mutations in *ARTEMIS, DNA-PKcs, CERNUNNOS, LIG4, NBS1, MRE11, ATM*

ADA, Adenosine deaminase; *AK2,* adenylate kinase 2; *ATM,* ataxia-telangiectasia mutated; *CRAC,* calcium release–activated channel; *DNA-PKcs,* DNA-dependent protein kinase catalytic subunit; *Ig,* immunoglobulin; *LIG4,* DNA ligase 4; *MRE11,* meiotic recombination homologue 11; *NBS1,* Nijmegen breakpoint syndrome 1; *NK cells,* natural killer cells; *PNP,* purine nucleoside phosphorylase; *SCID,* severe combined immunodeficiency; *TCR,* T cell receptor.

[a] Hypomorphic mutations in *RAG* genes and in *ARTEMIS* can cause Omenn syndrome.

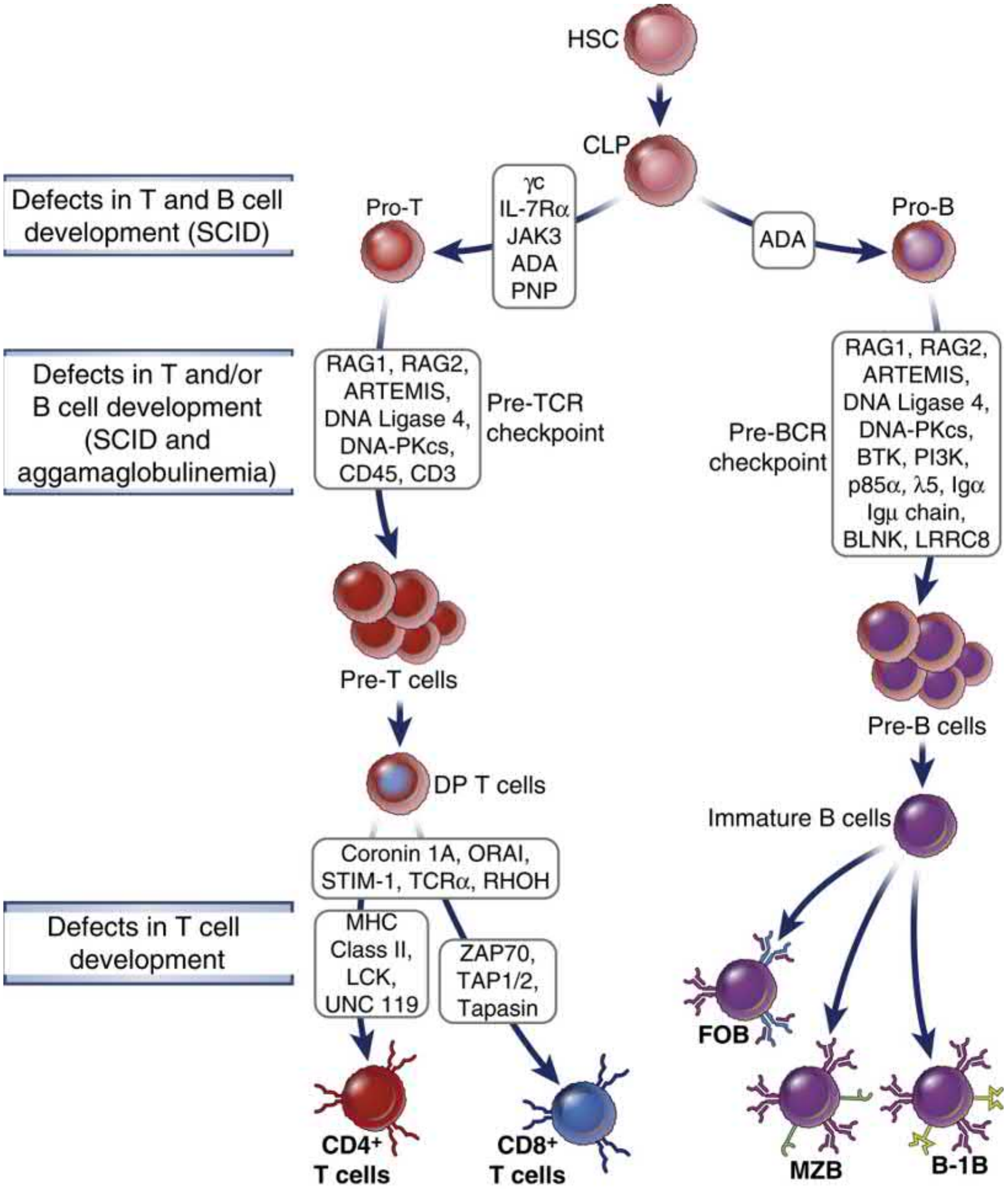

HSC
CLP
Defects in T and B cell development (SCID)
Pro-T
γc
IL-7Rα
JAK3
ADA
PNP
ADA
Pro-B
Defects in T and/or B cell development (SCID and aggamaglobulinemia)
RAG1, RAG2, ARTEMIS, DNA Ligase 4, DNA-PKcs, CD45, CD3
Pre-TCR checkpoint
Pre-BCR checkpoint
RAG1, RAG2, ARTEMIS, DNA Ligase 4, DNA-PKcs, BTK, PI3K, p85α, λ5, Igα Igμ chain, BLNK, LRRC8
Pre-T cells
Pre-B cells
DP T cells
Immature B cells
Coronin 1A, ORAI, STIM-1, TCRα, RHOH
Defects in T cell development
MHC Class II, LCK, UNC 119
ZAP70, TAP1/2, Tapasin
FOB
CD4+ T cells
CD8+ T cells
MZB
B-1B

FIG 21.1 Immunodeficiency caused by defects in B and T cell maturation.Primary immunodeficiencies caused by genetic defects in lymphocyte maturation are shown. Genes encoding the listed proteins are mutated in immunodeficient patients. These defects may affect T cell maturation alone, B cell maturation alone, or both. *ADA,* Adenosine deaminase; *B-1B,* B-1 B cells; *BCR,* B cell receptor; *CLP,* common lymphoid progenitor; *DP,*

double-positive; *FOB,* follicular B cells; *HSC,* hematopoietic stem cell; *IL,* interleukin; *MHC,* major histocompatibility complex; *MZB*, marginal zone B cells; *PNP,* purine nucleoside phosphorylase; *SCID,* severe combined immunodeficiency.

ADA Deficiency and Other Forms of SCID Caused by Defects in Nucleotide Metabolism

The most common cause of autosomal recessive SCID is deficiency of an enzyme called adenosine deaminase (ADA) due to mutations in the ADA gene. ADA functions in the salvage pathway of purine synthesis and catalyzes the irreversible deamination of adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively. Deficiency of the enzyme leads to the accumulation of deoxyadenosine and its precursors S-adenosylhomocysteine and deoxyadenosine triphosphate (dATP). These by-products have many toxic effects, including inhibition of DNA synthesis. Although ADA is present in most cells, developing lymphocytes are less efficient than most other cell types at degrading dATP into 2′-deoxyadenosine, and therefore lymphocyte maturation is particularly sensitive to ADA deficiency. Other features of the disease can include deafness, costochondral abnormalities, liver damage, and behavioral problems. ADA deficiency leads to reduced numbers of B and T cells; lymphocyte cell numbers are usually normal at birth but fall off precipitously during the first year of life. A few patients may have a nearly normal number of T cells, but these cells do not proliferate in response to antigenic stimulation.

A rarer autosomal recessive form of SCID is due to the deficiency of purine nucleoside phosphorylase (PNP), an enzyme that is also involved in purine catabolism. PNP catalyzes the conversion of inosine to hypoxanthine and guanosine to guanine, and deficiency of PNP leads to the accumulation of deoxyguanosine and deoxyguanosine triphosphate, with toxic effects on immature lymphocytes, mainly T cells. Autoimmune hemolytic anemia and progressive neurologic deterioration are also features of this disorder.

A particularly severe form of SCID is seen in a disease called **reticular dysgenesis.** This rare disorder is characterized by the absence of T and B lymphocytes and most myeloid cells, including granulocytes, and is due to a defect in the development of lymphoid and myeloid progenitors. This is an autosomal recessive disease due to a mutation in the adenylate kinase 2 *(AK2)* gene. The AK2 protein regulates the level of adenosine diphosphate, and in the absence of AK2 there is increased apoptosis of lymphoid and myeloid precursors.

Autosomal Recessive Mutations in Cytokine Signaling Components

Some patients with a disease clinically identical to X-linked SCID show an autosomal recessive inheritance. These patients have mutations affecting the IL-7 receptor α chain or the JAK3 kinase, which associates with the γc chain and is required for signaling by this receptor (see Chapter 7). Patients with mutations in the gene encoding the IL-7Rα chain have a defect in T cell development but exhibit normal NK cell development because IL-15 signaling is unaffected, and they have normal numbers of B cells.

Severe Combined Immunodeficiency Caused by Defects in V(D)J Recombination and Pre-TCR Checkpoint Signaling

Absence of V(D)J recombination leads to a failure to express the pre–TCR and the pre-B cell receptor (BCR) and a block in T and B cell development. Mutations in the *RAG1* or *RAG2* genes, which encode proteins that mediate the cleavage step during V(D)J recombination, or the *ARTEMIS* gene, which encodes an endonuclease that resolves coding-end hairpins during V(D)J recombination, all result in a failure of V(D)J recombination. These diseases are rare, but they account for a large percentage of the autosomal recessive forms of SCID. The functions of these proteins are discussed in Chapter 8. In children with these mutations, B and T lymphocytes are absent and immunity is severely compromised. Mutations in genes encoding proteins involved in double-stranded break repair or nonhomologous end-joining of DNA also lead to SCID because of defects in V(D)J recombination. These include genes encoding the catalytic subunit of the DNA-dependent protein kinase (DNA-PK) and DNA LIGASE 4. Genetic defects in this end-joining process also result in increased cellular sensitivity to radiation and can result in other manifestations, such as microcephaly, facial dysmorphisms, and defective tooth development.

Although most autosomal recessive forms of SCID are linked to mutations in *ADA, RAG1, RAG2,* and *ARTEMIS,* other forms of this syndrome are caused by mutations in the genes encoding the CD45 phosphatase (which is a positive regulator of SRC family kinases, such as FYN, LCK, and LYN) and mutations in the CD3 δ or ε chains or in the CD3-associated ζ chain. These mutations contribute to defective pre-TCR signaling and result in a block in αβ T cell development.

A specific defect in αβ T cell development and a clinical presentation that involves recurrent viral infections is caused by homozygous mutations in the gene encoding the T cell receptor α chain (TCRα) constant region. Affected individuals present with increased susceptibility to infections, including chronic varicella zoster and EBV infections, as well as autoimmunity and features of atopy. Clinical features include eosinophilia, vitiligo, eczema, alopecia areata, autoimmune hemolytic anemia, and the presence of other autoantibodies. The immune dysregulation may reflect the absence of regulatory T cells. The only T cells present in infants with this disease are γδ T cells.

Autosomal recessive mutations in *LCK,* which encodes a tyrosine kinase required for pre-TCR and TCR signaling, also cause a form of SCID with T cell deficiency, the lack of regulatory T cells, recurrent infections, and features of immune dysregulation.

Hypomorphic mutations (that only partially reduce function) in the *RAG* genes or in *ARTEMIS* are the cause of a disorder called

Omenn syndrome, characterized by reduced generation of T and B cells, immunodeficiency, and autoimmune and allergic manifestations. Omenn syndrome is phenotypically very different from SCID that is caused by more severe mutations in these very same genes, because the immunodeficiency in this syndrome coexists with exaggerated immune activation and autoimmunity. Although the mechanism underlying the excessive immune activation in this disease is unclear, it may be the result of an abnormally low ratio of regulatory T cells to effector T cells, or in cases with decreased V(D)J recombination, defective receptor editing in immature B cells. SCID caused by complete loss of RAG function and Omenn syndrome caused by partial loss illustrate how the severity of defects in the same gene can greatly influence the resulting phenotype.

DiGeorge Syndrome and Other Forms of SCID Due to Defective Thymic Epithelial Development

Complete or partial failure of development of the thymic anlage can lead to defective T cell maturation. The most common defect in thymic development causing SCID is seen in children with **DiGeorge syndrome.** This selective T cell deficiency results from defective development of the thymus and the parathyroid glands, as well as of other structures that arise from the third and fourth pharyngeal pouches during fetal life. The disease is characterized by immunodeficiency because of hypoplasia or agenesis of the thymus leading to deficient T cell maturation, abnormal calcium homeostasis and muscle twitching (tetany) due to an absence of parathyroid glands, abnormal development of the great vessels, and facial deformities. Different patients may show varying degrees of these abnormalities. The disease is caused most frequently by a deletion in the chromosomal region 22q11. A mouse line that has a similar defect in thymic development carries a mutation in a gene encoding a transcription factor called T-box 1 (TBX1), which lies within the same chromosomal region. This suggests that the immunodeficiency associated with DiGeorge syndrome can be explained, at least in part, by deletion of the *TBX1* gene.

In this syndrome, peripheral blood T lymphocytes are absent or greatly reduced in number, and the cells do not respond to polyclonal T cell activators or in mixed leukocyte reactions. Antibody levels are usually normal but may be reduced in severely affected patients. As in other severe T cell deficiencies, patients are susceptible to mycobacterial, viral, and fungal infections. The immunodeficiency associated with DiGeorge syndrome can be corrected by fetal thymic transplantation or by bone marrow transplantation. However, such treatment is usually not necessary, because T cell function tends to improve with age in a large fraction of patients and is often normal by 5 years. This improvement is probably because of some residual thymic tissue or because some as yet undefined extrathymic sites assume the function of T cell maturation. It is also possible that as these patients grow older, thymus tissue develops at ectopic sites (i.e., other than the normal location).

Autosomal recessive *FOXN1* mutations have been described in a small number of patients who present with SCID, alopecia (hair loss), and nail dystrophy. FOXN1 is a transcription factor of the Forkhead family that is required for the development of the thymic anlage and other ectodermal structures. The nude mouse, a strain that has been widely used in immunology research, lacks a thymus and hair because of a mutation in the same gene.

Apart from defects in the thymic anlage, genes that regulate egress of T cells from the thymus can also cause SCID. A rare defect in the thymus has been described involving a mutation in *CORONIN1A,* which encodes a protein that regulates the actin cytoskeleton. The absence of functional CORONIN1A results in defective egress of mature T cells from the thymus. Homozygous mutations in the *MST1* gene, which encodes a serine/threonine protein kinase, result in the failure of T cells to emigrate from the thymus and loss of naive T cells in the circulation. Patients present with recurrent bacterial and viral infections, and some develop EBV-driven lymphomas. Some patients present with epidermodysplasia verruciformis, a disorder characterized by HPV-infected warts and skin carcinomas. MST1 plays diverse roles in proliferation, cell survival, and cell migration. Although the major defect is in emigration of T cells from the thymus, there are also humoral immune defects in some patients who present with diminished B cell numbers and hypogammaglobulinemia.

Bare Lymphocyte Syndrome and Other Defects in T Cell Positive Selection

The generation of single-positive $CD4^+$ and $CD8^+$ T cells from double-positive thymocytes depends on positive selection and lineage commitment events. Specific inherited mutations in genes that regulate the process of positive selection abrogate the development of $CD4^+$ T cells or of $CD8^+$ T cells.

Class II major histocompatibility complex (MHC) deficiency, called **bare lymphocyte syndrome,** is a rare heterogeneous group of autosomal recessive diseases in which patients express little or no HLA (human leukocyte antigen)-DP, HLA-DQ, or HLA-DR on B lymphocytes, macrophages, and DCs and fail to express class II MHC molecules in response to IFN-γ. They express normal or only slightly reduced levels of class I MHC molecules. Most cases of bare lymphocyte syndrome are due to mutations in genes encoding proteins that regulate class II MHC gene transcription. For example, mutations affecting the constitutively expressed transcription factor RFX5 or the IFN-γ–inducible transcriptional activator CIITA lead to reduced class II MHC expression. Failure of antigen presentation may result in defective positive selection of T cells in the thymus, leading to a reduction in the number of mature $CD4^+$ T cells, and defective activation of surviving $CD4^+$ cells in the periphery. Affected individuals are deficient in DTH responses and in antibody responses to T cell–dependent protein antigens. The disease appears within the first year of life and is usually fatal unless it is treated by hematopoietic stem cell transplantation.

Autosomal recessive class I MHC deficiencies have also been described and are characterized by decreased $CD8^+$ T cell numbers and function. In some cases, the failure to express class I MHC molecules is due to mutations in the *TAP-1* or *TAP-2* genes, which encode the subunits of the TAP (transporter associated with antigen processing) complex. TAP normally transports peptides from the cytosol into the endoplasmic reticulum, where they are loaded onto class I MHC molecules (see Chapter 6). Because empty MHC molecules are degraded intracellularly, the level of cell surface class I MHC molecules is reduced in these TAP-deficient patients, a phenotype similar to *tap* gene knockout mice. Such patients suffer mainly from necrotizing granulomatous skin lesions and respiratory tract bacterial infections, but not viral infections, which is surprising considering that a principal function of $CD8^+$ T cells is defense against viruses. A similar deficiency of class I MHC expression has been observed in patients with mutations in the gene encoding the TAPASIN protein, which is required for loading the peptides transported into the ER onto nascent class I MHC molecules (see Chapter 6).

Patients with ZAP70 deficiency have a lineage commitment defect resulting in reduced $CD8^+$ T cells but normal numbers of $CD4^+$ T cells; the reason for the selective loss is not clear. Although $CD4^+$ T cell development or emigration to the periphery is not compromised, the cells fail to proliferate normally when challenged with antigens.

SCID Caused by Defective T Cell Activation

Another rare form of SCID is caused by a mutation in the gene encoding ORAI1, a component of the CRAC (calcium release–activated calcium) channel (see Chapter 7). Antigen receptor signaling leads to the activation of the γ isoform of phospholipase C (PLCγ) and the inositol trisphosphate (IP3)-dependent release of calcium ions from the endoplasmic reticulum and mitochondria. The released calcium is replenished by CRAC channels that facilitate an influx of extracellular calcium. This process is crucial for lymphocyte activation, and it is defective in cells with mutant *ORAI1*. A similar phenotype is observed in patients with mutations in *STIM1,* which encodes an endoplasmic reticulum protein that senses the depletion of calcium stores and contributes to the opening of the CRAC channel. Patients with *ORAI1* and *STIM1* mutations do not exhibit a defect in T cell development, but their T cells cannot be properly activated.

Antibody Deficiencies: Defects in B Cell Development and Activation

Whereas defects in T cell development or in both T and B cell development cause the SCID phenotype, more circumscribed defects in B cells result in disorders in which the primary abnormality is in antibody production (Table 21.4). Some of these disorders are caused by defects in B cell development (see Fig. 21.1), and others are caused by abnormal B cell activation and antibody synthesis (Fig. 21.2). In one subset of hyper-IgM syndromes, discussed later, antibody deficiencies are also accompanied by defects in macrophage and APC activation, which, in turn, result in defective cell-mediated immunity.

X-Linked Agammaglobulinemia: An X-Linked Pre-BCR Signaling Defect

X-linked agammaglobulinemia, also called Bruton's agammaglobulinemia, is caused by mutations or deletions in the gene encoding an enzyme called Bruton tyrosine kinase (BTK), resulting in a failure of B cells to mature beyond the pre-B cell stage in the bone marrow (see Fig. 21.1) **.** The disease is characterized by the absence of antibodies (gamma globulins) in the blood, as the name implies. It is one of the most common congenital immunodeficiencies and the prototype of a failure of B cell maturation. BTK is involved in transducing signals from the pre-BCR that are required for the survival and differentiation of pre-B cells (see Chapter 8). In female carriers of this disease, the only mature B cells are those that have inactivated the X chromosome carrying the mutant allele. Patients with X-linked agammaglobulinemia usually have low or undetectable serum Ig, reduced or absent B cells in peripheral blood and lymphoid tissues, no germinal centers in lymph nodes, and no plasma cells in tissues. The maturation, numbers, and functions of T cells are generally normal, although some studies have revealed reduced numbers of activated T cells in patients, which may be a consequence of reduced antigen presentation caused by the lack of B cells. Autoimmune disorders such as arthritis develop in almost 20% of patients; whether this is related to failure of self-tolerance or is a consequence of chronic infections because of the immunodeficiency has not been established. BTK is also involved in the activation of myeloid cells, and susceptibility to infection, in addition to reflecting the absence or near absence of antibodies, could also result in part from defective innate immune function. The infectious complications of X-linked agammaglobulinemia are greatly reduced by periodic (e.g., weekly or monthly) injections of IgG from healthy adult donors. Such preparations contain preformed antibodies against common pathogens and provide effective passive immunity.

Autosomal recessive forms of agammaglobulinemia have been described, most of which can be linked to defects in pre-BCR signaling. Mutant genes that have been identified in this context include genes encoding the μ (IgM) heavy chain, the $\lambda 5$ surrogate light chain, Igα (a signaling component of the pre-BCR and BCR), the p85α subunit of PI3 kinase, and BLNK (an adaptor protein downstream of the pre-BCR and BCR).

Selective Immunoglobulin Isotype Deficiencies

Many immunodeficiencies that selectively involve one or a few Ig isotypes have been described. The most common is **selective IgA deficiency,** which affects approximately 1 in 700 individuals of Caucasian descent, and is thus the most common primary immunodeficiency in North America and Europe. IgA deficiency usually occurs sporadically, but many familial cases with either autosomal dominant or autosomal recessive patterns of inheritance are also known. The clinical features are variable. Many people with

IgA deficiency are normal; others have occasional respiratory infections and diarrhea; and, rarely, patients have severe, recurrent infections leading to permanent intestinal and airway damage, with associated autoimmune disorders. These manifestations reflect the importance of secretory IgA in protection of mucosal barriers from commensal and pathogenic microbes (see Chapter 14). IgA deficiency is characterized by low serum IgA, usually less than 50 μg/mL (normal, 2 to 4 mg/mL), with normal or elevated levels of IgM and IgG and low IgA in mucosal secretions. The disease is genetically heterogeneous, and in some patients there is a defect in IgA class switching, though the underlying basis for this defect has not been identified. In a small proportion of patients with selective IgA deficiency, mutations have been described in *TACI* (transmembrane activator and calcium modulator and cyclophilin ligand interactor), one of the three types of receptors for the cytokines BAFF (B cell–activating factor) and APRIL (a proliferation-inducing ligand), both of which stimulate B cell survival and proliferation. *TACI* mutations are also a cause of common variable immunodeficiency (CVID), discussed later.

Table 21.4

Disease	Functional Deficiencies	Mechanism of Defect
Agammaglobulinemias		
X-linked	Decrease in all serum Ig isotypes; reduced B cell numbers	Pre-B receptor checkpoint defect; *BTK* mutation
Autosomal recessive forms	Decrease in all serum Ig isotypes; reduced B cell numbers	Pre-B receptor checkpoint defect; mutations in IgM heavy chain (μ), surrogate light chains *(λ5), Igα, BLNK, PI3K p85α*
Hypogammaglobulinemias/Isotype Defects		
Selective IgA deficiency	Decreased IgA; may be associated with increased susceptibility to bacterial infections and protozoa such as *Giardia lamblia*	Mutations in *TACI* in some patients
Selective IgG2 deficiency	Increased susceptibility to bacterial infections	Small subset have deletion in IgH γ2 locus
Common variable immunodeficiency	Hypogammaglobulinemia; normal or decreased B cell numbers	Mutations in *ICOS*, *TACI*, *CTLA4*, and many other genes have been identified in some patients
ICF syndrome	Hypogammaglobulinemia; occasional mild T cell defects	Mutations in *DNMT3B*
Hyper-IgM Syndromes		
X-linked	Defects in T helper cell–mediated B cell, macrophage, and dendritic cell activation; defects in somatic mutation, class switching, and germinal center formation; defective cell-mediated immunity	Mutation in *CD40L*
Autosomal recessive with cell-mediated immune defects	Defects in T helper cell–mediated B cell, macrophage, and dendritic cell activation; defects in somatic mutation, class switching, and germinal center formation; defective cell-mediated immunity	Mutations in *CD40, NEMO*
Autosomal recessive with antibody defect only	Defects in somatic mutation and isotype switching	Mutations in *AID, UNG*

AID, Activation-induced cytidine deaminase; *BTK,* Bruton's tyrosine kinase; *DNMT3B,* DNA methyltransferase 3B; *ICF,* immunodeficiencies-centromeric instability-facial anomalies; *ICOS,* inducible costimulator; *Ig,* immunoglobulin; *NEMO,* NF-κB essential modulator; *TACI,* transmembrane activator and calcium modulator and cyclophilin ligand interactor; *UNG,* uracil N-glycosylase.

Selective IgG subclass deficiencies have been described in which total serum IgG levels are normal but concentrations of one or more subclasses are below normal. IgG3 deficiency is the most common subclass defect in adults, and IgG2 deficiency associated with reduced IgA is most common in children. Some individuals with these deficiencies have recurrent bacterial infections, but many do not have any clinical problems. Selective IgG subclass deficiencies are usually due to abnormal B cell differentiation and rarely to homozygous deletions of various constant region ($C\gamma$) genes.

Defects in B Cell Differentiation: Common Variable Immunodeficiency (CVID)

CVID is a group of heterogeneous disorders defined by reduced levels of serum Ig, impaired antibody responses to infection and vaccines, and increased incidence of infections. It is the most common immunodeficiency seen in adolescents and young adults. The diagnosis is usually one of exclusion when other primary immunodeficiency diseases are ruled out. The presentation and pathogenesis are, as the name implies, highly variable. The diagnosis is made based on very low serum IgG levels, decreased IgM and/or IgA, and poor antibody response to vaccines, with known genetic causes of hypogammaglobulinemia being ruled out. The prevalence in Caucasian populations is estimated to be between 1 in 10,000 and 1 in 50,000. Although Ig deficiency and associated pyogenic infections, typically with *Haemophilus influenzae* and *S. pneumoniae,* are major features of these disorders, autoimmune diseases, including pernicious anemia, hemolytic anemia, inflammatory bowel disease, and rheumatoid arthritis, may be just as significant clinically. A high incidence of malignant tumors, particularly lymphomas, is also associated with CVID. These disorders may be diagnosed early in childhood or late in life. The majority of cases are sporadic, but from 5% to 25% of patients have a family history. Monogenic forms of CVID exhibit mainly autosomal dominant inheritance, although some patients show autosomal recessive inheritance. Mature B lymphocytes are present, but memory B cells are typically reduced in the blood and plasma cells are absent in lymphoid tissues, which suggests a block in B cell differentiation. The defective antibody production has been attributed to multiple abnormalities, including intrinsic B cell defects or deficient T cell help.

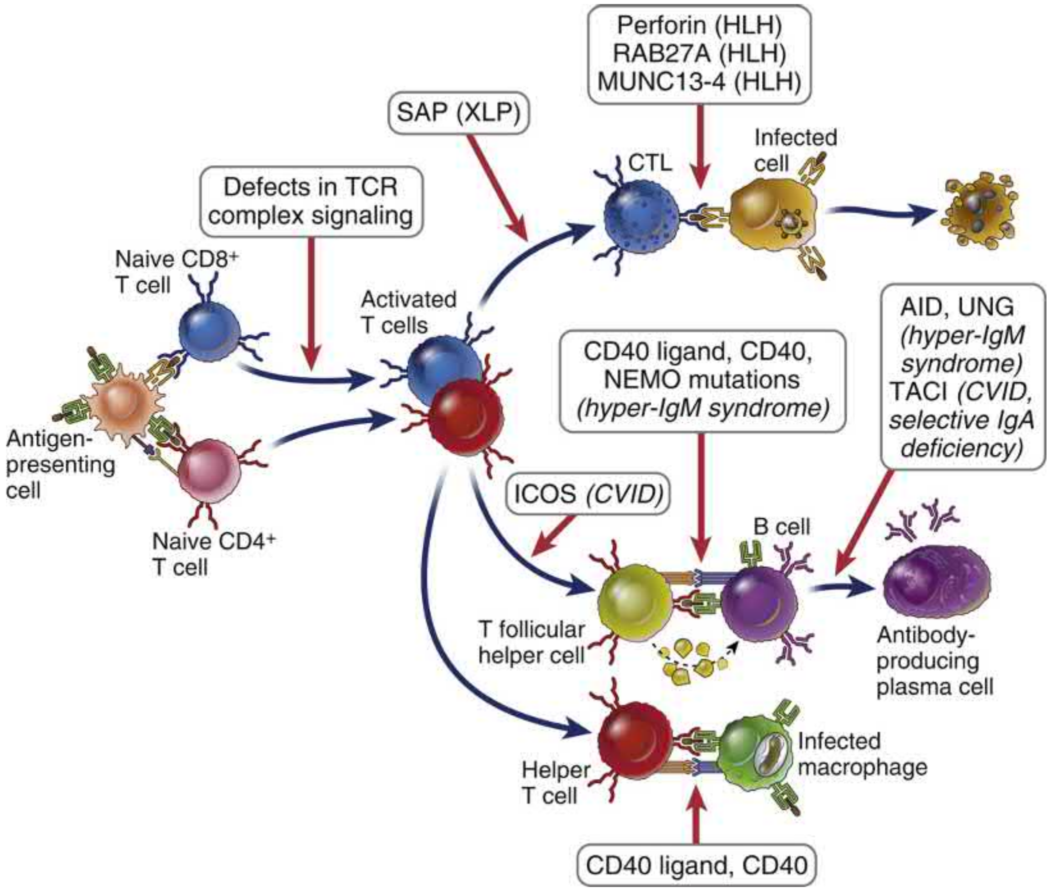

Perforin (HLH)
RAB27A (HLH)
MUNC13-4 (HLH)
SAP (XLP)
Infected cell
CTL
Defects in TCR complex signaling
Naive CD8+ T cell
Activated T cells
AID, UNG (hyper-IgM syndrome) TACI (CVID, selective IgA deficiency)
CD40 ligand, CD40, NEMO mutations (hyper-IgM syndrome)
Antigen-presenting cell
ICOS (CVID)
B cell
Naive CD4+ T cell
T follicular helper cell
Antibody-producing plasma cell
Infected macrophage
Helper T cell
CD40 ligand, CD40

FIG 21.2 Immunodeficiency caused by defects in B and T cell activation. Primary immunodeficiencies may be caused by genetic defects in molecules required for T or B lymphocyte antigen receptor signaling, for helper T cell–mediated activation of B cells and macrophages (and dendritic cells), or for activation and function of cytotoxic T lymphocytes *(CTLs)* (and natural killer cells). Genes encoding the listed proteins are mutated in immunodeficient patients. *AID,* Activation-induced cytidine deaminase; *CVID,* common variable immunodeficiency; *HLH,* hemophagocytic lymphohistiocytosis; *ICOS,* inducible costimulator; *IgM,* immunoglobulin M; *TACI,* transmembrane activator and CAML interactor; *TCR,* T cell receptor; *UNG,* uracil N-glycosylase; *XLP,* X-linked lymphoproliferative disease.

Monogenic causes account for approximately 10% of cases of CVID. So far, mutations in over 25 different genes have been shown to be associated with CVID. Mutations in *TACI*, described earlier in the context of selective IgA deficiency, have been described in some cases. Activating mutations in *PIK3CD,* the gene encoding the catalytic subunit of PI3 kinase δ, lead to hyperactivation of T cells and a block in B cell development that results in CVID. Loss-of-function mutations in *CTLA4* result in defective function of regulatory T cells and a poorly explained block in B cell development, which also causes CVID. A small proportion of patients with CVID have a mutation in the *ICOS* (inducible T cell costimulator) gene. ICOS is required for T follicular helper (Tfh) cell generation (see Chapter 12). A few cases of CVID have been linked to mutations in the *CD19* gene. CD19 is a signaling component of the CR2 (CD21) coreceptor complex (see Chapter 7). Many other gene mutations have been identified in different CVID patients. Even in patients in whom a mutated gene is identified, the inheritance pattern is often more complex than in usual Mendelian diseases.

Defects in T Cell–Dependent B Cell Activation: Hyper-IgM Syndromes

The X-linked hyper-IgM syndrome is caused by mutations in the gene encoding the T cell effector molecule CD40 ligand (CD154). It is a rare disorder associated with defective switching of B cells to the IgG and IgA isotypes; production of these antibodies is therefore reduced, and the major isotype detected in the blood is IgM. Production of high-affinity antibodies is also impaired. The mutant forms of CD40 ligand produced in these patients fail to transduce signals through CD40 and therefore do not stimulate B cells to undergo heavy chain isotype switching, which requires engagement of CD40 on B cells by CD40L on helper T cells (see Chapter 12). Patients suffer from infections similar to those seen in other Ig deficiencies. Patients with X-linked hyper-IgM syndrome also show defects in cell-mediated immunity, with an increased susceptibility to infection by the intracellular fungal microbe *P. jiroveci*. This defective cell-mediated immunity occurs because CD40 ligand and CD40 are also involved in T cell–dependent activation of macrophages and DCs (see Chapter 10). Knockout mice lacking CD40 or CD40 ligand have a phenotype similar to that of the human disease.

A small subset of cases of hyper-IgM syndrome show an autosomal recessive inheritance pattern. In these patients, the genetic defects may be in CD40 or in the enzyme activation–induced deaminase (AID), which is involved in heavy chain isotype switching and affinity maturation (see Chapter 12). An even rarer form of hyper-IgM syndrome is caused by autosomal recessive mutations in the gene encoding uracil N-glycosylase (UNG; see Chapter 12), an enzyme that removes U residues from Ig genes during class switching and somatic mutation. An inherited disorder, EDA-ID, in which hypomorphic *NEMO* mutations contribute to a hyper-IgM state and defects in ectodermal structures, is described earlier in the section on defects in innate immunity.

AID and *UNG* mutations affect class-switch recombination and somatic hypermutation in distinct ways. In the absence of AID, both switching and hypermutation are defective because AID is required for both processes. In the absence of UNG, isotype switching is defective but somatic hypermutation is largely preserved, although it exhibits less A:T mutations than normal. Nevertheless, the largely shared phenotypes of mutations affecting CD40L, CD40, AID, and UNG link all these genes in one pathway of B cell activation, an excellent example of how genetic diseases inform our understanding of biologic mechanisms. Predictably, mutations affecting AID and UNG affect B cell responses but, unlike CD40 or CD40L mutations, do not compromise cell-mediated immunity.

Defects in T Lymphocyte Activation and Function

Congenital abnormalities in the activation of T lymphocytes are being increasingly recognized as our understanding of the molecular basis of lymphocyte activation improves (Table 21.5). Included in this broad category are some disorders of CTL and NK cell granule composition or exocytosis. Although we classify disorders linked to defective MHC expression with disorders of T cell development, these abnormalities also result in defective activation of T cells that do mature and emerge from the thymus.

Defects in TCR Signal Transduction

Many rare immunodeficiency diseases are caused by defects in the expression of molecules required for T cell activation and function. Biochemical and molecular analyses of affected individuals have revealed mutations in the genes encoding various T cell proteins (see Table 21.5). Examples include impaired TCR complex expression or function caused by mutations in the *CD3* ε or γ genes or defective TCR-mediated signaling caused by mutations in *ZAP70*. These defects are often found in only a few isolated cases or in a few families, and the clinical features and severity vary widely. Patients with these abnormalities may have deficiencies predominantly in T cell function or have mixed T cell and B cell immunodeficiencies despite normal or even elevated numbers of blood lymphocytes. We have previously mentioned the impact of mutations affecting the CD3 complex, LCK, and other proteins on the development of all T cells; the effect of *ZAP70* mutations on $CD8^+$ T cell development; the effect of *LCK* and *UNC119* mutations on $CD4^+$ T cell development; and the relevance of *ORAI1* and *STIM1* mutations in T cell activation, all in the clinical context of SCID. Some other syndromes involving the defective activation of mature T cells are considered here.

Hyper-IgE Syndromes

The hyper-IgE syndromes (HIESs), also known as Job syndrome, represent a collection of primary immunodeficiency syndromes in which patients have eczema, eosinophilia, recurrent pulmonary infections, and staphylococcal and fungal skin abscesses. The older name, Job syndrome, was based on the biblical description: “So went Satan forth from the presence of the Lord, and smote Job with sore boils from the sole of his foot unto the crown.” One autosomal dominant form of HIES results from heterozygous dominant-negative mutations affecting STAT3, a transcription factor that is critical for cell responses to IL-6, IL-10, IL-17, IL-21, and IL-22. Defective STAT3 signaling results in diminished acute-phase responses downstream of IL-6, defective innate immunity and defective Th17 responses, and this likely results in the propensity for *Staphylococcus aureus*–mediated cold abscesses (see Chapter 10). The defect in STAT3 has many pleiotropic effects beyond the effect on Th17 cells and also contributes to defective inhibitory responses mediated by IL-10. Another autosomal recessive cause of HIES is mutation in the gene encoding DOCK8, a guanine nucleotide exchange factor. *DOCK8* mutations result in reduced numbers of T cells, B cells, and NK cells and defects in lymphocyte signaling and cytoskeletal rearrangements resembling those seen in the Wiskott-Aldrich syndrome. DOCK8 participates in a pathway leading to ARP2/3 complex–mediated actin polymerization and is also required for the maintenance of STAT3 phosphorylation. The name of this syndrome emphasizes increased levels of IgE in the blood; the basis of this abnormality is likely linked to defective JAK-STAT signaling leading to an increase in Tfh cells that make IL-4 and IL-13, which drive IgE responses.

X-Linked Lymphoproliferative Disease

X-linked lymphoproliferative disease (XLP) is a disorder characterized by an inability to eliminate EBV, eventually leading to fulminant infectious mononucleosis and the development of B cell lymphoma. In approximately 80% of cases, the disease is due to mutations in the gene encoding an adaptor molecule called SLAM (signaling lymphocyte activation molecule)-associated protein (SAP) that binds to a family of cell surface molecules involved in the activation of NK cells and T and B lymphocytes. SAP links the membrane proteins SLAM and 2B4 (see Chapter 7) to the SRC family kinase FYN. Defects in SAP contribute to attenuated NK and T cell activation and result in increased susceptibility to viral infections. As discussed in Chapter 12, SAP is required for Tfh cell development, and the inability of XLP patients to produce germinal centers and high-affinity antibodies also likely contributes to the associated hypogammaglobulinemia and susceptibility to viral infection. In approximately 20% of cases of XLP, the genetic defect resides not in SAP but in the gene encoding XIAP (X-linked inhibitor of apoptosis). The resulting enhanced apoptosis of T cells and NK-T cells leads to a marked depletion of these cell types. This immunodeficiency is most commonly manifested by severe opportunistic EBV infections.

Table 21.5

Disease	Functional Deficiencies	Mechanism of Defect
Defective T Cell Signaling		
Proximal TCR signaling defects	Defects in cell-mediated immunity and T-cell–dependent humoral immunity	Mutations in *CD3* genes, *CD45, STIM1, ORAI1*
Wiskott-Aldrich syndrome **Autosomal recessive WAS-like disease**	Defective T cell activation and leukocyte mobility Defective T cell activation and leukocyte mobility	TCR-dependent actin-cytoskeletal rearrangements are defective because of mutations in *WASP,* or less often the WASP-interacting protein gene *WIP*
Hyper-IgE syndromes	Defective Th17 and ILC3 cells	Mutations in *STAT3, DOCK8*
Familial Hemophagocytic Lymphohistiocytoses		
X-linked lymphoproliferative disease	Uncontrolled EBV-induced B cell proliferation, uncontrolled macrophage and CTL activation, defective NK cell and CTL function	Mutations in *SAP* Mutations in *XIAP*
X-linked immunodeficiency-magnesium defects-EBV infection-neoplasia syndrome	Uncontrolled EBV viremia and lymphoma	Mutations in *MAGT1*
Perforin deficiencies	Uncontrolled macrophage and CTL activation, defective NK cell and CTL function	Mutations in *PERFORIN*
Granule fusion	Uncontrolled macrophage and CTL activation, defective NK cell and CTL function	Defective cytotoxic granule exocytosis; mutations in *RAB27A, MUNC13-4, SYNTAXIN, AP3* (and in *LYST* in Chédiak-Higashi syndrome—see Table 21.2)

AP3, Adaptor-related protein complex 3; *CTL,* cytotoxic T lymphocyte; *EBV,* Epstein-Barr virus; *IgE,* immunoglobulin E; *ILC3,* innate lymphocyte cell 3; *LYST,* lysosomal trafficking regulator protein; *MHC,* major histocompatibility complex; *NK cell,* natural killer cell; *SAP,* SLAM-associated protein; *TAP,* transporter associated with antigen processing; *TCR,* T cell receptor; *WASP,* Wiskott-Aldrich syndrome protein; *WIP,* WASP-interacting protein; *XIAP,* X-linked inhibitor of apoptosis.

X-Linked Immunodeficiency-Magnesium Defects-EBV Infection-Neoplasia Syndrome

Mutations in the gene on the X chromosome encoding MAGT1 (magnesium transporter protein 1) result in a defect in NK cell and CTL function and also contribute to CD4$^+$ T cell lymphopenia. Patients have recurrent EBV and other infections and lymphomas. This disorder is known as the XMEN (X-linked immunodeficiency-magnesium defects-EBV infection-neoplasia) syndrome. Intracellular levels of free magnesium that are induced during T cell and NK cell activation by MAGT1 contribute to the activation of PLCγ1 in these cells and help to mediate subsequent calcium signaling. The cell signaling defect can be restored by supplementing the diet with magnesium. B cells (which have high levels of a different PLCγ isoform, PLCγ2) are unaffected by the absence of this transporter. This disorder highlights the role of magnesium in T cell activation.

Defective CTL and NK Cell Function: Familial Hemophagocytic Lymphohistiocytosis

Familial hemophagocytic lymphohistiocytosis (HLH) syndromes are a group of life-threatening immunodeficiency disorders in which NK cells and CTLs are defective in their ability to kill infected cells. As a result, viral infections are not held in check, and compensatory excessive macrophage activation is a feature of these syndromes. Because of this feature, another name for these diseases is **macrophage activation syndrome.** A late but striking feature of these disorders is the ingestion of red blood cells by activated macrophages (hemophagocytosis). Mutations in the *perforin* gene are the most common cause of familial HLH, but mutations in genes encoding the cellular machinery involved in granule exocytosis are found in some cases of this syndrome. Specifically, mutations affecting RAB27A, a small guanosine triphosphatase involved in vesicular fusion, and MUNC13-4, an adaptor that participates in granule exocytosis, compromise the fusion of lytic granules with the plasma membrane and thus contribute to various subtypes of HLH. Similarly, mutations in the gene for one component of the AP3 cytosolic adaptor protein complex can also disrupt intracellular transport and contribute to a form of HLH. It is thought that T cells and NK cells have defective cytotoxic activity and cannot clear intracellular infections, and the excessive compensatory IFN-γ–mediated macrophage activation is manifested by hemophagocytosis and lymphadenopathy in the context of immunodeficiency. An antibody that neutralizes IFN-γ is approved for the treatment of familial macrophage activation syndromes.

Multisystem Disorders With Immunodeficiency

Immunodeficiency is often one of a constellation of symptoms in a number of inherited disorders that affect multiple organs.

Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome is an X-linked disease characterized by eczema, thrombocytopenia (reduced blood platelets), and susceptibility to bacterial infections. Some of the abnormalities in this disorder can be traced to defective T cell activation, although intrinsic loss of B cell function also contributes to the pathogenesis. In the initial stages of the disease, lymphocyte numbers are normal, and the principal defect is an inability to produce antibodies in response to T cell–independent polysaccharide antigens, because of which these patients are particularly susceptible to infections with encapsulated bacteria. The lymphocytes (and platelets) are smaller than normal. With increasing age, the patients show reduced numbers of lymphocytes and more severe immunodeficiency.

The gene that is mutated in Wiskott-Aldrich syndrome encodes a cytoplasmic protein called WASP (Wiskott-Aldrich syndrome protein), which is expressed exclusively in bone marrow–derived cells. WASP interacts with several proteins, including adaptor molecules downstream of the antigen receptor such as GRB2 (see

Chapter 7), the ARP2/3 complex involved in actin polymerization, and small G proteins of the RHO family that regulate actin cytoskeletal rearrangement. Defective formation of immune synapses between T cells and APCs, resulting in poor activation of the lymphocytes, and impaired mobility of all leukocytes may account for the immunodeficiency observed in this syndrome. An autosomal recessive disease that resembles Wiskott-Aldrich syndrome has been described. This disease is caused by mutations in the gene encoding WIP (WASP-interacting protein), a protein that binds to WASP and stabilizes it.

Ataxia-Telangiectasia

Ataxia-telangiectasia is an autosomal recessive disorder characterized by abnormal gait (ataxia), vascular malformations (telangiectases), neurologic deficits, increased incidence of tumors, and immunodeficiency. The disease is caused by mutations in a gene that encodes a protein kinase called ATM (ataxia-telangiectasia mutated). The immunologic defects are of variable severity and may affect both B and T cells. ATM is required for double strand (ds) DNA break repair that is relevant to both V(D)J recombination and class switch recombination, as described next. The most common humoral immune defects are IgA and IgG2 deficiency, probably because of the crucial role of ATM in class-switch recombination. The T cell defects, which are usually less pronounced, are associated with thymic hypoplasia. Patients experience upper and lower respiratory tract bacterial infections, multiple autoimmune phenomena, and increasingly frequent cancers with advancing age.

ATM is related structurally to PI3 kinase and activates cell cycle checkpoints and apoptosis in response to dsDNA breaks. It also has been shown to contribute to the stability of double-strand DNA break complexes during V(D)J recombination. In ataxia-telangiectasia, these abnormalities in DNA repair account for abnormal generation of antigen receptors. In addition, ATM contributes to DNA stability when dsDNA breaks are generated in the course of Ig class switch recombination, and mutations in *ATM* result in defective class switching and reduced levels of IgG, IgA, and IgE.

Therapeutic Approaches for Primary Immunodeficiencies

Treatments for immunodeficiencies have two aims: to minimize and control infections and to replace the defective or absent components of the immune system by antibody replacement or hematopoietic stem cell transplantation. Passive immunization with pooled gamma globulin is very beneficial for agammaglobulinemic patients and has been lifesaving for many boys with X-linked agammaglobulinemia. Hematopoietic stem cell transplantation is currently the treatment of choice for many primary immunodeficiency diseases and has been successful in the treatment of SCID, Wiskott-Aldrich syndrome, bare lymphocyte syndrome, and LADs. Enzyme replacement therapy for ADA deficiency is a common treatment. Injection of bovine ADA, conjugated to polyethylene glycol to prolong its serum half-life, has proved successful in some cases, but the benefits are usually short-lived.

In theory, the ideal therapy for congenital disorders of lymphocytes is to replace the defective gene in self-renewing stem cells. Gene replacement has proved successful for some immunodeficiency disorders. The main obstacles to this type of gene therapy remain difficulties in purifying self-renewing stem cells, which are the ideal target for introduction of the replacement gene, and in introducing genes into cells to achieve stable, long-lived, and high-level expression. In addition, transplant recipients have to be conditioned by depleting their bone marrow cells to allow transplanted stem cells to engraft, and this carries potential risks because of transient reduction of blood cells. Considerable progress has been made in gene therapy for ADA deficiency and X-linked SCID by use of a mild conditioning approach. Patients with

X-linked SCID have been successfully treated by transplantation of autologous bone marrow cells engineered to express a normal γc gene. In the early trials, a small number of the treated patients developed leukemia, apparently because the introduced γc gene inserted adjacent to an oncogene and activated that gene. The development of newer self-inactivating lentiviral vectors has reduced the risk for insertional mutagenesis, and this has led to successful gene therapy for both ADA-SCID and X-linked SCID.

Table 21.6

Secondary (Acquired) Immunodeficiencies

Cause	Mechanism
HIV infection	Depletion of $CD4^+$ T cells
Protein-calorie malnutrition	Metabolic derangements inhibit lymphocyte maturation and function
Irradiation and chemotherapy for cancer	Decreased bone marrow lymphocyte precursors
Cancer metastases and leukemia involving bone marrow	Reduced site of leukocyte development
Immunosuppression for transplants, autoimmune diseases	Reduced lymphocyte activation, cytokine blockade, impaired leukocyte trafficking
Loss of the spleen as a result of trauma, sickle cell disease, or surgery	Decreased phagocytosis of blood-borne bacteria

Secondary (Acquired) Immunodeficiencies

Deficiencies of the immune system often develop because of abnormalities that are not genetic but acquired during life (Table 21.6) **.** Acquired immunodeficiency diseases are, in fact, more common than congenital immunodeficiencies and are caused by a variety of pathogenic mechanisms. First, immunosuppression may occur as a biologic complication of another disease process. Second, so-called iatrogenic immunodeficiencies may develop as complications of therapy for other diseases. Third, immunodeficiency may result from an infection that target cells of the immune system. The most prominent of these is HIV infection, which is described separately later in the chapter.

Disorders in which immunodeficiency is a frequent complicating element include malnutrition, cancer, and infections. Protein-calorie malnutrition is common in lower-income countries and is associated with impaired cellular and humoral immunity to microorganisms. Much of the morbidity and mortality that afflict malnourished people is due to infections. The basis for the immunodeficiency is not well defined, but it is reasonable to assume that the global metabolic disturbances in these individuals, caused by deficient intake of protein, fat, vitamins, and minerals, will adversely affect maturation and function of the cells of the immune system.

Patients with advanced widespread cancer often are susceptible to infection because of impaired cell-mediated and humoral immune responses to a variety of microbes. Bone marrow tumors, including cancers metastatic to marrow and leukemias that arise in the marrow, may interfere with the growth and development of normal lymphocytes and other leukocytes. In addition, tumors may produce substances that interfere with lymphocyte development or function.

Various types of infections lead to immunosuppression. Viruses other than HIV are known to impair immune responses; examples include the measles virus and HTLV-1 (human T cell lymphotropic virus 1). Both viruses can infect lymphocytes, which may be a basis for their immunosuppressive effects. Like HIV, HTLV-1 is a retrovirus with tropism for $CD4^+$ T cells; however, instead of killing helper T cells, HTLV-1 transforms them and produces an aggressive malignant neoplasm called adult T cell leukemia/lymphoma (ATL). Patients with ATL typically have severe immunosuppression with multiple opportunistic infections. Chronic infections with *Mycobacterium tuberculosis* and various fungi frequently result in anergy to many antigens. Chronic parasitic infections may also lead to immunosuppression. For example, African children with chronic malarial infections have depressed T cell function, and this may be one reason why these children have an increased propensity to develop EBV-associated malignant tumors.

Iatrogenic immunosuppression is most often due to drug therapies that kill or functionally inactivate lymphocytes or block the function of cytokines made by innate immune cells and lymphocytes. Some drugs are given intentionally to immunosuppress patients, either for the treatment of inflammatory diseases or to prevent rejection of organ allografts. The most commonly used antiinflammatory and immunosuppressive drugs are corticosteroids and calcineurin inhibitors, respectively, but many others, including anticytokine antibodies, are widely used now (see Chapters 17 and 19). Various chemotherapeutic drugs are administered to patients with cancer, and these drugs are usually cytotoxic to proliferating cells, including mature and developing lymphocytes as well as other leukocyte precursors. Thus, cancer chemotherapy is almost always accompanied by a period of immunosuppression and increased risk for infection. Iatrogenic immunosuppression and tumors involving the bone marrow are the most common causes of immunodeficiency in higher-income countries.

Another form of acquired immunodeficiency results from the absence of a spleen caused by surgical removal of the organ after trauma and as treatment of certain hematologic diseases such as autoimmune hemolytic anemia and thrombocytopenia, in which red cells and platelets, respectively, are destroyed by phagocytes in the spleen, or infarction in sickle cell disease. Patients without spleens are more susceptible to infection by some organisms, particularly bacteria such as pneumococci and meningococci, which have polysaccharide-rich capsules and are normally cleared by opsonization and phagocytosis. This enhanced susceptibility is partly due to defective phagocytic clearance of the microbes in the blood, an important function of the spleen, and partly due to defective antibody responses resulting from the absence of marginal zone B cells.

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

AIDS is the disease caused by infection with HIV and is characterized by profound immunosuppression with associated opportunistic infections and malignant tumors, wasting, and neurocognitive disorders. HIV primarily infects activated CD4$^+$ helper T cells. Macrophages and DCs can also harbor the virus but do so less efficiently than activated CD4$^+$ T cells. HIV emerged as a human pathogen recently relative to most other known human pathogens, and the HIV epidemic was first identified only in the 1980s. However, the degree of morbidity and mortality caused by HIV and the global impact of this infection on health care resources and economies are already enormous and continue to grow. HIV has infected 70 to 80 million people and has caused the death of more than 34 million adults and children. Approximately 38 million people are living with HIV infection and AIDS, of which approximately 70% are in Africa and 20% in Asia, and almost a million people die of the disease every year. An especially devastating feature of the disease is that half of the approximately 3 million new cases every year occur in young adults (15 to 24 years of age). AIDS has left approximately 14 million orphans. About 1.7 million children under 15 have HIV and more than 90% of these children acquired AIDS from their mothers during fetal life, childbirth, or while breastfeeding. About 100,000 children die of AIDS every year. Currently, there is no vaccine or permanent cure for AIDS, but antiretroviral drugs are in use that are very effective at controlling the infection. In this section of the chapter, we describe the properties of HIV, the pathogenesis of HIV-induced immunodeficiency, and the clinical and epidemiologic features of HIV-related diseases.

An Overview of HIV Virology

HIV is a member of the lentivirus family of animal retroviruses. Lentiviruses, including visna virus of sheep and the bovine, feline, and simian immunodeficiency viruses, are capable of long-term latent infection of cells and short-term cytopathic effects, and they all produce slowly progressive, fatal diseases that include wasting syndromes and CNS abnormalities. Two closely related types of HIV, designated HIV-1 and HIV-2, have been identified. HIV-1 is by far the most common cause of AIDS; HIV-2, which differs in genomic structure and antigenicity, causes a form of AIDS with slower progression than HIV-1–linked disease and is mainly confined to West Africa.

HIV Structure and Genes

An infectious HIV particle consists of two identical strands of RNA packaged within a core of viral proteins and surrounded by a phospholipid bilayer envelope derived from the host cell membrane but including virally encoded membrane proteins (Fig. 21.3). The RNA genome of HIV is approximately 9.2 kb long and has the basic arrangement of nucleic acid sequences characteristic of all known retroviruses (Fig. 21.4). Long terminal repeats (LTRs) at each end of the genome regulate viral gene expression, viral integration into the host genome, and viral replication. The *gag* sequence encodes core structural proteins. The *env* sequence encodes the HIV Envelope (Env) protein. Env exists as a trimer of three molecules of a glycoprotein, gp120, and a stem of three molecules of gp41 that is inserted in the viral lipid bilayer. The *pol* sequence encodes reverse transcriptase, integrase, and viral protease enzymes, which are required for viral replication. In addition to these typical retrovirus genes, the HIV-1 genome contains six other regulatory genes unique to HIV, namely, the *tat, rev, vif, nef, vpr,* and *vpu* genes, whose products regulate viral replication and host immune evasion in various ways. The functions of these genes are summarized in Fig. 21.4 and discussed later.

Viral Life Cycle

HIV infection of cells begins when the envelope glycoprotein gp120 of the virus binds to two proteins on host cells, CD4 and usually a chemokine receptor that functions as a coreceptor (Fig. 21.5) **.** The viral particles that initiate infection are usually in the blood, semen, or other body fluids of one individual and are introduced into another individual by sexual contact, needle stick, or transplacental passage. The two components of Env, the transmembrane gp41 subunit and the external, noncovalently associated gp120 subunit, are produced by proteolytic cleavage of a gp160 precursor. The trimeric Env complex mediates a multistep process of fusion of the virion envelope with the membrane of the target cell (Fig. 21.6). The first step of this process is the binding of gp120 subunits to CD4 molecules, which induces a conformational change that promotes secondary gp120 binding to a chemokine receptor, generally CCR5 or CXCR4, which functions as a coreceptor for the virus. HIV binding to the coreceptor induces a conformational change in gp41, its refolding into a six-helix bundle that exposes a hydrophobic region, called the fusion peptide, which then inserts into the cell membrane and enables the viral membrane to fuse with the target cell membrane. Fusion may occur at the cell surface or in a macropinocytic or endosomal compartment. After the virus completes its life cycle in the infected cell (described later), free viral particles are released from the infected cell and bind to an uninfected cell, thus propagating the infection. In addition, gp120 and gp41, which are expressed on the plasma membrane of infected cells before virus is released, can mediate cell-cell fusion with an uninfected cell that expresses CD4 and coreceptors, and HIV genomes can then be passed between the fused cells directly.

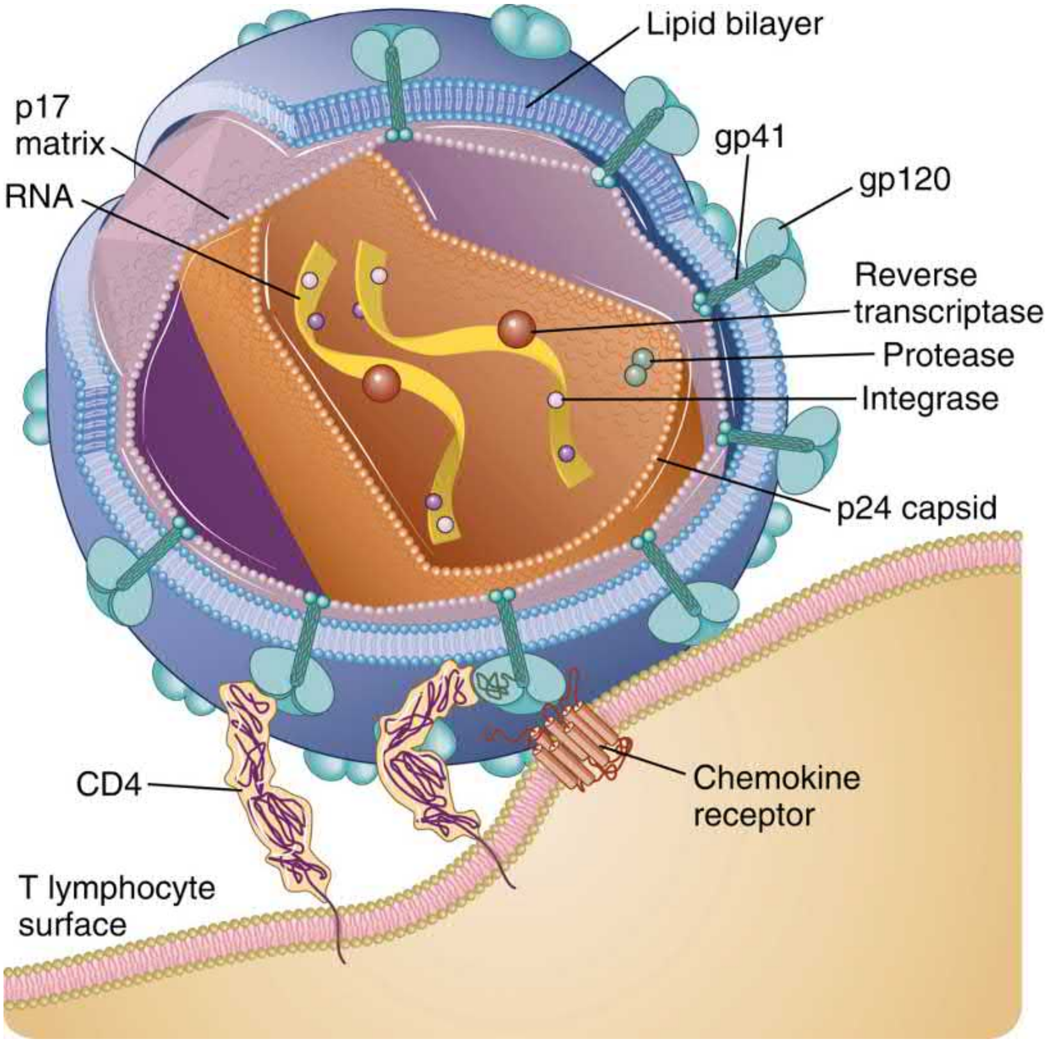

Lipid bilayer
p17 matrix
RNA
gp41
gp120
Reverse transcriptase
Protease
Integrase
p24 capsid
CD4
Chemokine receptor
T lymphocyte surface

FIG 21.3 Structure of HIV-1.An HIV-1 virion is shown next to a T cell surface. HIV-1 consists of two identical strands of RNA (the viral genome) and associated enzymes, including reverse transcriptase, integrase, and protease, packaged in a cone-shaped core composed of p24 capsid protein with a surrounding p17 protein matrix, all surrounded by a phospholipid membrane envelope derived from the host cell. Virally encoded membrane proteins (gp41 and gp120) are bound to the envelope. CD4 and chemokine receptors on the host cell surface function as HIV-1 receptors.

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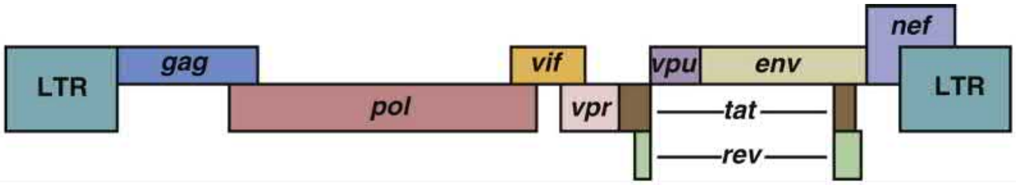


LTR	Transcription of viral genome; integration of viral DNA into host cell genome; binding site for host transcription factors
gag	Nucleocapsid core and matrix proteins
pol	Reverse transcriptase, protease, integrase, and ribonuclease
env	Viral coat proteins (gp120 and gp41)
vif	Overcomes inhibitory effect of host cell enzyme (APOBEC3G), promotes viral replication
vpr	Increases viral replication and suppresses host mRNA splicing
tat	Required for elongation of viral transcripts
rev	Promotes nuclear export of partially spliced viral RNAs
vpu	Downregulates host cell CD4 expression; enhances release of virus from cells; counteracts host restriction factor tetherin
nef	Downregulates host cell CD4 and class I MHC expression; modulates intracellular signaling to facilitate viral replication; also counteracts tetherin

FIG 21.4 HIV-1 genome.The genes along the linear genome are indicated as differently colored blocks. Some genes use some of the same sequences as other genes, as shown by overlapping blocks, but are read differently by host cell RNA polymerase. The coding sequences of the *tat* and *rev* genes are separated in the genome and require RNA splicing to produce functional mRNA. *env,* envelope; *gag,* group-specific antigen; *LTR,* long terminal repeat; *nef,* negative effector; *pol,* polymerase; *rev,* regulator of viral gene expression; *tat,* transcriptional activator; *vif,* viral infectivity factor; *vpr,* viral protein R; *vpu,* viral protein u.

Modified from Greene W. *AIDS and the immune system. Sci Am.* 1993;269[3], 98–105. Copyright © 1993 by Scientific American, Inc.

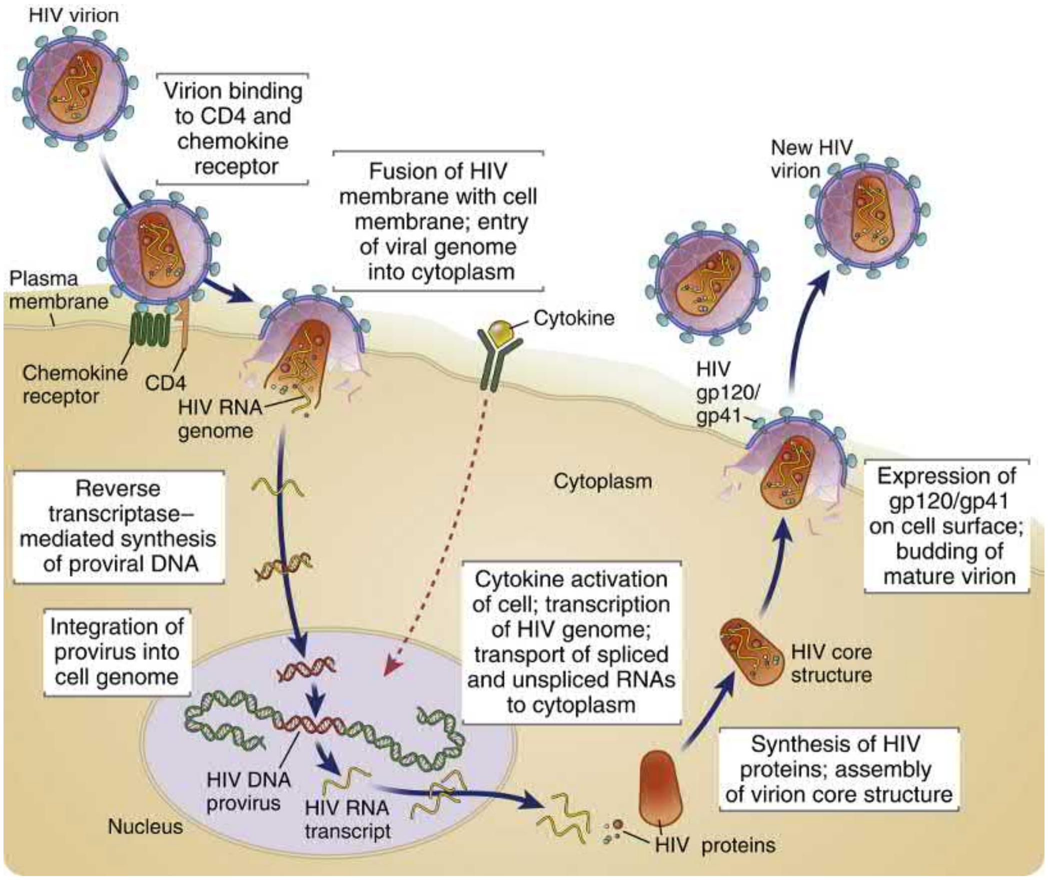

HIV virion
Virion binding to CD4 and chemokine receptor
Fusion of HIV membrane with cell membrane; entry of viral genome into cytoplasm
New HIV virion
Plasma membrane
Cytokine
Chemokine receptor
CD4
HIV RNA genome
HIV gp120/ gp41
Cytoplasm
Expression of gp120/gp41 on cell surface; budding of mature virion
Reverse transcriptase–mediated synthesis of proviral DNA
Integration of provirus into cell genome
Cytokine activation of cell; transcription of HIV genome; transport of spliced and unspliced RNAs to cytoplasm
HIV core structure
Synthesis of HIV proteins; assembly of virion core structure
HIV DNA provirus
HIV RNA transcript
Nucleus
HIV proteins

FIG 21.5 HIV life cycle. The sequential steps in the life cycle of HIV are shown, from initial infection of a host cell to viral replication and release of a new virion. For the sake of clarity, the production and release of only one new virion are shown. An infected cell actually produces many virions, each capable of infecting cells, thereby amplifying the infectious cycle. Proviral transcription is activated by cytokines or antigen (not shown).

The most important chemokine receptors that act as coreceptors for HIV are CCR5 and CXCR4. More than seven different chemokine receptors have been shown to serve as coreceptors for HIV entry into cells, and several other proteins belonging to the seven-transmembrane–spanning G protein–coupled receptor family, such as the leukotriene B_4 receptor, can also mediate HIV infection of cells. Different isolates of HIV and indeed different

HIV strains within the same individual have distinct tropisms for different cell populations.

Although CD4$^+$ T cells are the main cellular target of HIV, some strains of HIV that have Env proteins that possess a very high affinity for CD4 can infect macrophages as well; these strains are sometimes called macrophage (M)-tropic strains. The density of CD4 on macrophages is 20 times lower than it is on CD4$^+$ T cells, and the expression on the macrophage cell surface of CCR5 and CXCR4 is also considerably lower than the levels of these proteins on CD4$^+$ T cells.

Although genetically distinct viruses exist in any infected individual, typically only a single viral strain is successfully transmitted from an infected individual to a previously uninfected one. The transmitted virus is called the founder strain. Founder strains that are transmitted from one person to another have a relatively low affinity for CD4 and exclusively use the CCR5 molecule as a coreceptor. These strains, called T-tropic strains, enter macrophages about 30 times less efficiently than M-tropic strains. Person-to-person transmission almost always involves only T-tropic strains. The importance of CCR5 in HIV infection in vivo is supported by the finding that individuals who do not express this receptor on the cell surface because of an inherited homozygous deletion in the *CCR5* gene are resistant to HIV infection.

After an HIV virion enters a cell, the enzymes within the nucleoprotein complex become active and begin the viral reproductive cycle (see Fig. 21.5) **.** The nucleoprotein core of the virus becomes disrupted, the RNA genome of HIV is reverse-transcribed into a dsDNA by viral reverse transcriptase, and the viral DNA enters the nucleus. The viral integrase also enters the nucleus and catalyzes the integration of viral DNA into the host cell genome. The integrated HIV DNA is called the provirus. The provirus may remain transcriptionally inactive for months or years, with little or no production of new viral proteins or virions, and in this way HIV infection of an individual cell can be latent. The contribution of latent viruses to the HIV reservoir is discussed later.

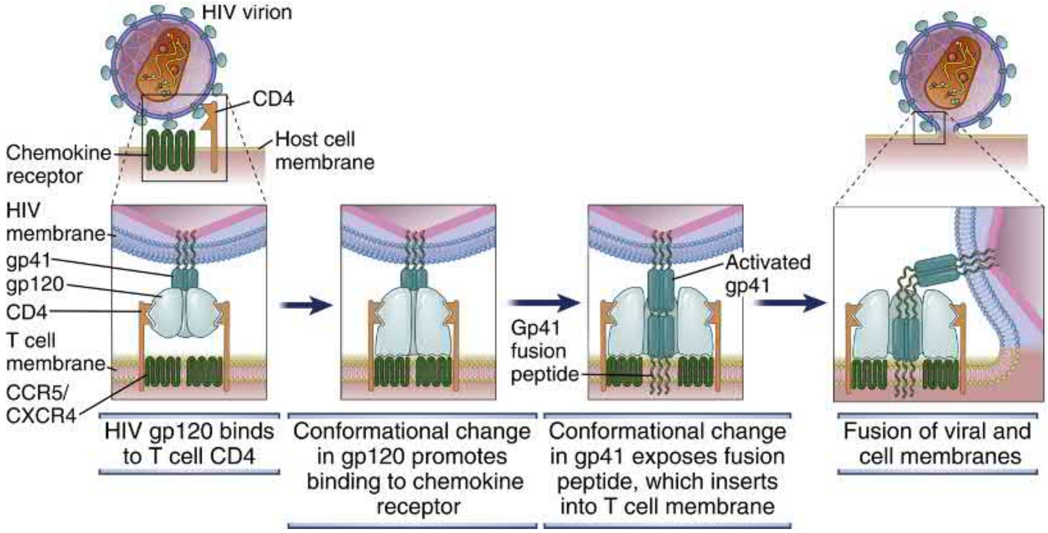

HIV virion
CD4
Chemokine receptor
Host cell membrane
HIV membrane
gp41
gp120
CD4
T cell membrane
CCR5/ CXCR4
Activated gp41
Gp41 fusion peptide
HIV gp120 binds to T cell CD4
Conformational change in gp120 promotes binding to chemokine receptor
Conformational change in gp41 exposes fusion peptide, which inserts into T cell membrane
Fusion of viral and cell membranes

FIG 21.6 Mechanism of HIV entry into a cell. In the model depicted, sequential conformational changes in gp120 and gp41 are induced by binding to CD4. These changes promote binding of the virus to the coreceptor (a chemokine receptor) and fusion of the HIV-1 and host cell membranes. The fusion peptide of activated gp41 contains hydrophobic amino acid residues that mediate insertion into the host cell plasma membrane.

Transcription of the genes of the integrated DNA provirus is regulated by the LTR upstream of the viral structural genes, and cytokines and other stimuli that activate T cells and macrophages enhance viral gene transcription. The LTRs contain polyadenylation signal sequences, the TATA box promoter sequence, and binding sites for two host cell transcription factors, NF-κB and SP1. Initiation of HIV gene transcription in T cells is linked to activation of the T cells by antigen or cytokines. For example, polyclonal activators of T cells, such as phytohemagglutinin, and cytokines, such as IL-2, tumor necrosis factor (TNF), and lymphotoxin, stimulate HIV gene expression in infected T cells; various macrophage-activating cytokines, including IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF), stimulate HIV gene expression and viral replication in infected monocytes and macrophages. TCR and cytokine stimulation of HIV gene transcription probably involves the activation of NF-κB and its binding to sequences in the viral LTR. This phenomenon is significant to the pathogenesis of AIDS because the normal response of a latently infected T cell to a microbe may be the way in which HIV latency is ended and virus production begins.

The Tat protein is required for HIV gene expression and acts by enhancing the production of complete viral mRNA transcripts. Even in the presence of optimal signals to initiate transcription, few if any HIV mRNA molecules are actually synthesized without the action of Tat because transcription of HIV genes by mammalian RNA polymerase is inefficient, and the polymerase complex usually stops before the mRNA is completed. Tat allows DNA-dependent RNA polymerase to remain bound to the viral DNA molecule long enough for transcription to be completed and to thus produce a functional viral mRNA.

Synthesis of mature infectious viral particles begins after full-length viral RNA transcripts are produced and the viral genes are expressed as proteins. The mRNAs encoding the various HIV proteins are derived from a single full-genome-length transcript by differential splicing events. HIV gene expression may be divided into an early stage, during which regulatory genes are expressed, and a late stage, during which structural genes are expressed and full-length viral genomes are packaged. The Rev, Tat, and Nef proteins are early gene products encoded by fully spliced mRNAs that are exported from the nucleus and translated into proteins in the cytoplasm soon after infection of a cell. Late genes include *env*, *gag*, and *pol*, which encode the structural components of the virus and are translated from singly spliced or unspliced RNA. The Rev protein initiates the switch from early to late gene expression by promoting the export of these incompletely spliced late gene RNAs out of the nucleus. The *pol* gene product is a precursor protein that is sequentially cleaved to form reverse transcriptase, protease, ribonuclease, and integrase enzymes. As mentioned earlier, reverse transcriptase and integrase proteins are required to produce a DNA copy of the viral RNA genome and to integrate it as a provirus into the host genome. The *gag* gene encodes a 55-kD protein that is proteolytically cleaved into the p24 capsid protein, p17 matrix protein, p7 nucleocapsid protein, p6 domain, and two spacer peptides, sp1 and sp2, by the action of the viral protease encoded by the *pol* gene. These *gag*-derived peptides and proteins are required for the assembly of infectious viral particles. The primary product of the *env* gene is a 160-kD glycoprotein (gp160) that is cleaved by cellular proteases within the endoplasmic reticulum into the gp120 and gp41 proteins required for HIV binding to cells, as discussed earlier. Current antiviral drug therapy for HIV disease includes inhibitors of the enzymes reverse transcriptase, protease, and integrase.

After the transcription of various viral genes, viral proteins are synthesized in the cytoplasm. Assembly of infectious viral particles then begins by packaging full-length RNA transcripts of the proviral genome within a nucleoprotein complex that includes the gag core proteins and the *pol*-encoded enzymes required for the next cycle of integration. This nucleoprotein complex then buds from the plasma membrane, capturing Env and host glycoproteins as part of its envelope. The rate of virus production can reach sufficiently high levels to cause cell death, as discussed later.

Pathogenesis of HIV Infection and AIDS

HIV disease begins with acute infection, which is only partly controlled by the host immune response, and advances to chronic progressive infection of peripheral lymphoid tissues (Fig. 21.7) **.** The virus typically enters through mucosal epithelia. The subsequent events in the infection can be divided into several phases.

Acute (early) infection is characterized by infection of activated CD4$^+$ T cells in mucosal lymphoid tissues and the death of many infected CD4$^+$ T cells. A large number of activated and memory CD4$^+$ T cells reside at mucosal sites, primarily in the gastrointestinal tract, and can be infected by HIV. Within 2 to 4 weeks of infection, a large fraction of mucosal CD4$^+$ T cells may be destroyed.

The transition from the acute phase to the chronic phase of infection is accompanied by dissemination of the virus, viremia, and development of host adaptive immune responses. DCs in epithelia at sites of virus entry capture the virus and then migrate into the lymph nodes. DCs express a protein with a mannose-binding lectin domain, called DC-SIGN, which may be particularly important in binding the HIV envelope, transporting the virus, and mediating trans-infection of CD4$^+$ T cells. Once in lymphoid tissues, DCs may pass HIV on to CD4$^+$ T cells through direct cell-cell contact. Within days after the first exposure to HIV, viral replication can be detected in the lymph nodes and gut lymphoid tissues. This replication leads to viremia, during which large numbers of HIV particles are present in the patient's blood, accompanied by an acute HIV syndrome that includes a variety of nonspecific signs and symptoms typical of many viral infections (described later). The viremia allows the virus to disseminate throughout the body and to infect helper T cells, macrophages, and DCs in peripheral lymphoid tissues. As the HIV infection spreads, the adaptive immune system mounts both humoral and cell-mediated immune responses directed at viral antigens, which we will describe later. These immune responses partially control the infection and viral production, and such control is reflected by a drop in viremia to low but detectable levels by approximately 12 weeks after the primary exposure.

In the next, chronic, phase of the disease, lymph nodes, the spleen, and the gastrointestinal tract are sites of continuous HIV replication and cell destruction (see Fig. 21.7) **.** During this period of the disease, the immune system remains competent at handling most infections with opportunistic microbes, and few or no clinical manifestations of the HIV infection are present. Therefore, this phase of HIV disease is called the clinical latency period. Although the majority of peripheral blood T cells do not harbor the virus, destruction of $CD4^+$ T cells within lymphoid tissues steadily progresses during the latent period, and the number of circulating blood $CD4^+$ T cells steadily declines (Fig. 21.8). More than 90% of the body's approximately 10^{12} T cells are normally found in peripheral and mucosal lymphoid tissues, and it is estimated that HIV destroys up to 1 to 2×10^9 $CD4^+$ T cells every day. Early in the course of the disease, the individual may continue to make new $CD4^+$ T cells, and therefore these cells can be replaced almost as quickly as they are destroyed. At this stage, up to 10% of $CD4^+$ T cells in lymphoid organs may be infected, but the number of circulating $CD4^+$ T cells that are infected at any one time may be less than 0.1% of the total $CD4^+$ T cells in an individual. Eventually, over a period of years, the continuous cycle of virus infection, T cell death, and new infection leads to an inexorable loss of $CD4^+$ T cells from the lymphoid tissues and the circulation.

Mechanisms of Immunodeficiency Caused by HIV

HIV infection ultimately results in impaired function of both the adaptive and innate immune systems. The most prominent defects are in cell-mediated immunity, which result mainly from the destruction of $CD4^+$ T cells. Both infected and possibly noninfected $CD4^+$ T cells may be lost.

A major cause of the loss of $CD4^+$ T cells in HIV-infected individuals is the direct effect of infection of these cells by HIV. Death of $CD4^+$ T cells is associated with production of virus in infected cells and may contribute to the decline in the numbers of these cells. Several direct toxic effects of HIV on infected $CD4^+$ cells have been described.

- The process of virus production, with expression of gp41 in the plasma membrane and budding of viral particles, may lead to increased plasma membrane permeability and the influx of lethal amounts of calcium, which induces apoptosis, or osmotic lysis of the cell caused by the influx of water.
- Viral production can interfere with cellular protein synthesis and thereby lead to cell death.

Mechanisms in addition to virus-induced death of infected CD4 $^+$ T cells have been proposed for the depletion and functional impairment of these cells in HIV-infected individuals. One mechanism is related to chronic activation of uninfected cells by the infections that are common in patients infected with HIV and also by cytokines produced in response to these infections. Chronic activation of the T cells may predispose them to apoptosis; the molecular pathway involved in this type of activation-induced cell death is not yet defined specifically in the HIV context (in general, many activated CD4$^+$ T cells, especially Th1 cells, may be eliminated by FASL-FAS interactions). Apoptotic death of activated lymphocytes may account for the observation that the loss of T cells greatly exceeds the numbers of HIV-infected cells. As mentioned earlier, HIV-specific CTLs are present in many patients with AIDS, and these cells may kill infected CD4$^+$ T cells, and some abortively infected cells might die by pyroptosis. In addition, antibodies against HIV envelope proteins may bind to HIV-infected CD4$^+$ T cells and target the cells for ADCC. Binding of gp120 to newly synthesized intracellular CD4 may interfere with normal protein processing in the endoplasmic reticulum and block cell surface expression of CD4, making the cells incapable of responding to antigenic stimulation. The relative importance of these indirect mechanisms of CD4$^+$ T cell depletion in HIV-infected patients is uncertain and controversial.

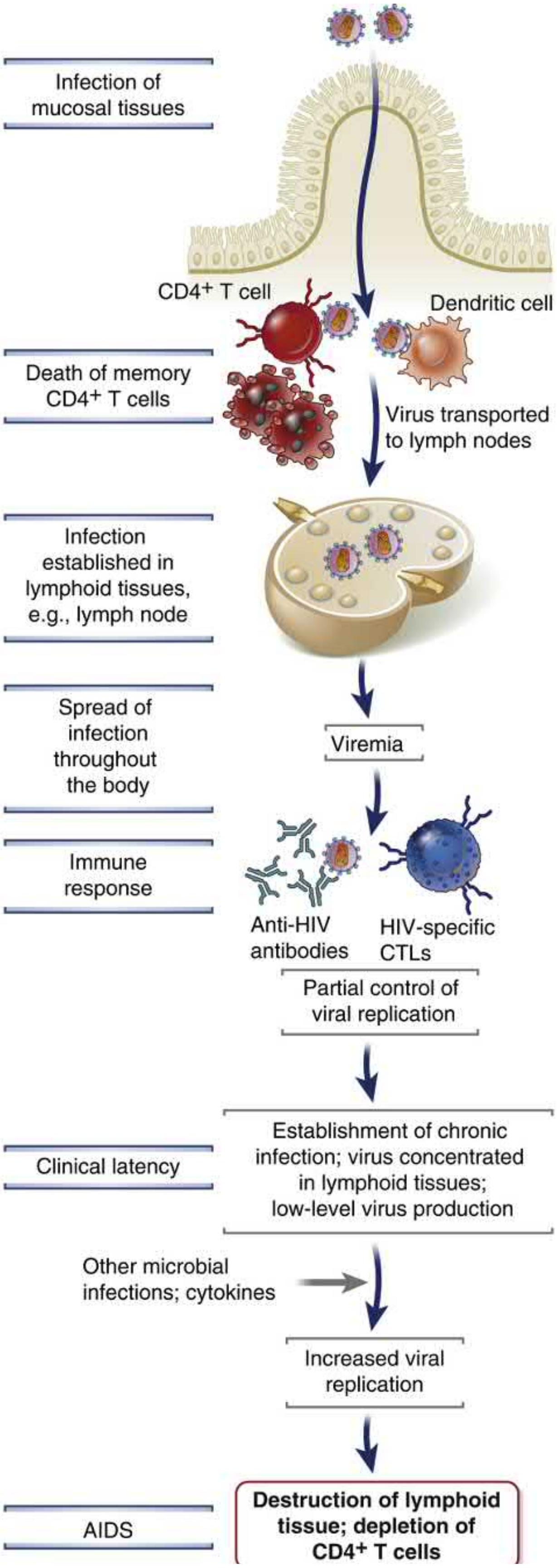

Infection of mucosal tissues
CD4+ T cell
Dendritic cell
Death of memory CD4+ T cells
Virus transported to lymph nodes
Infection established in lymphoid tissues, e.g., lymph node
Spread of infection throughout the body
Viremia
Immune response
Anti-HIV antibodies
HIV-specific CTLs
Partial control of viral replication
Clinical latency
Establishment of chronic infection; virus concentrated in lymphoid tissues; low-level virus production
Other microbial infections; cytokines
Increased viral replication
AIDS
Destruction of lymphoid tissue; depletion of CD4+ T cells

FIG 21.7 Progression of HIV infection. The progression of HIV infection correlates with spread of the virus from the initial site of infection to lymphoid tissues throughout the body. The immune response of the host temporarily controls acute infection but does not prevent the establishment of chronic infection of cells in lymphoid tissues. Cytokine stimuli induced by other microbes serve to enhance HIV production and progression to AIDS. *CTLs,* Cytotoxic T lymphocytes.

Functional defects in the immune system of HIV-infected individuals exacerbate the immune deficiency caused by depletion of CD4$^+$ T cells. These functional defects include a decrease in T cell responses to antigens and nonneutralizing and less vigorous humoral immune responses, even though total serum Ig levels may be elevated. The defects may be a result of the direct effects of HIV infection on CD4$^+$ T cells, including the effects of soluble gp120 released from infected cells binding to uninfected cells. For example, CD4 that has bound gp120 may not be available to interact with class II MHC molecules on APCs, and thus T cell responses to antigens would be inhibited. Also, gp120 binding to CD4 may deliver signals that downregulate helper T cell function. Some studies have demonstrated that patients with HIV infection have increased numbers of CD4$^+$CD25$^+$ regulatory T cells, but it is not yet clear if this is a consistent finding or if these cells actually contribute to defective immunity.

Macrophages, DCs, and follicular dendritic cells (FDCs) may be infected or injured by HIV, and the resulting abnormalities of these cells also contribute to the progression of immunodeficiency.

- Although macrophages are susceptible to HIV infection, they are relatively resistant to the cytopathic effects of HIV. Macrophages also may be infected by a gp120/gp41-

independent route, such as phagocytosis of the virus or other infected cells or Fc receptor–mediated endocytosis of antibody-coated HIV virions. Because macrophages can be infected but are not generally killed by HIV, they may become a reservoir for the virus. In fact, the quantity of macrophage-associated HIV exceeds T cell–associated virus in some tissues from patients with AIDS, notably the brain. HIV-infected macrophages may be impaired in antigen presentation functions and cytokine secretion.

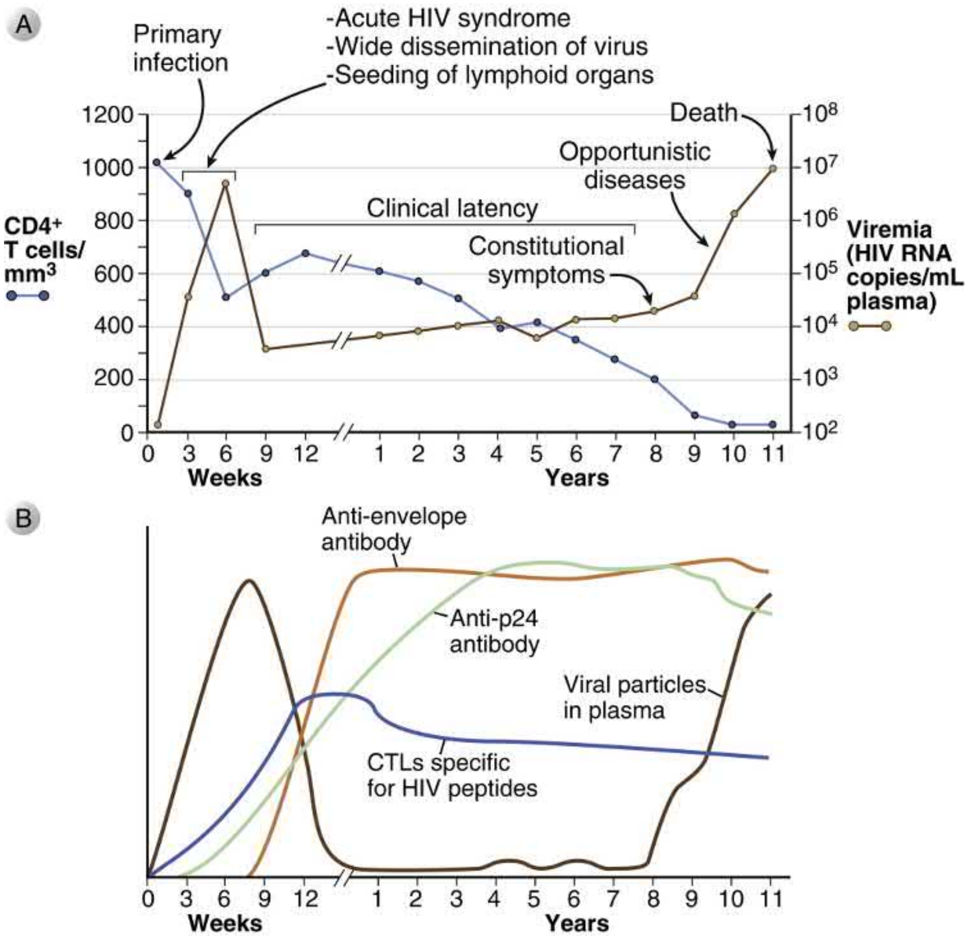


FIG 21.8 Clinical course of HIV disease. **A,** Plasma viremia, blood CD4⁺ T cell counts, and clinical stages of disease. Approximately 12 weeks after infection, the blood-borne virus (plasma viremia) is reduced to very low levels (detectable only by sensitive reverse transcriptase–polymerase chain reaction assays) and stays this way for many years. Nonetheless, CD4⁺ T cell counts steadily decline during this clinical latency period because of active viral replication and T cell infection in lymph nodes. When CD4⁺ T cell counts drop below a critical level (~200/mm^3), the risk for infection and other clinical features of AIDS is high. **B,** Immune response to HIV infection. A cytotoxic T

lymphocyte *(CTL)* response to HIV is detectable by 2 to 3 weeks after the initial infection and peaks by 9 to 12 weeks. Marked expansion of virus-specific CD8$^+$ T cells occurs during this time, and up to 10% of a patient's CTLs may be HIV specific at 12 weeks. The humoral immune response to HIV peaks at about 12 weeks.

A, From Pantaleo G, Graziosi C, Fauci AS. New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med*. 1993;328:327–335. Copyright 1993 by Massachusetts Medical Society.

- DCs can also capture HIV and can possibly also be infected. Like macrophages, DCs are not directly injured by HIV infection. However, these cells form intimate contact with naive T cells during the course of antigen presentation. It is proposed that DCs infect naive T cells during these encounters and this may be a pathway for spread of the infection.
- FDCs in the germinal centers of lymph nodes and the spleen trap large amounts of HIV on their surfaces, in part by Fc receptor–mediated binding of antibody-coated virus. Although FDCs are not efficiently infected, they contribute to the pathogenesis of HIV-associated immunodeficiency in at least two ways. First, the FDC surface is a reservoir for HIV that can infect macrophages and CD4$^+$ T cells in the lymph nodes. Second, the normal functions of FDCs in immune responses are impaired, and they may eventually be destroyed by the virus. Although the mechanisms of HIV-induced death of FDCs are not understood, the net result of loss of the FDC network in the lymph nodes and spleen is a profound dissolution of the architecture of the peripheral lymphoid system. Tfh cells in germinal centers

may also contribute to the HIV reservoir, discussed in the following section.

HIV Reservoirs and Viral Turnover

The virus detected in patients' blood is produced mostly by short-lived infected CD4$^+$ T cells and in smaller amounts by other infected cells. Three phases of decay of plasma viremia have been observed in patients treated with antiretroviral drugs or predicted by mathematical modeling, and these decay curves have been used to surmise the distribution of HIV in different cellular reservoirs. More than 90% of plasma virus is thought to be produced by activated and memory CD4$^+$ T cells that are major reservoirs and sources of the virus in infected patients. About two-thirds of the HIV reservoir exists in the mucosal immune system, mainly in the gastrointestinal tract, and about one-third is in lymph nodes. Although there are ongoing attempts to enhance host immunity and clear the virus completely from infected individuals (often referred to as an HIV cure), the resilience of the HIV reservoir has proved a confounding factor. Many latent viruses within the reservoir remain relatively protected from the host immune system. Latent viruses in Tfh cells in the light zones of germinal centers have been assumed to be protected from cytotoxic CD8$^+$ T cells. Some plasma virus may be produced by macrophages, which have a slower turnover (half-life of ~2 weeks). It is hypothesized that a very small fraction of the virus, perhaps as little as 1%, is present in latently infected memory T cells. Because of the long life span of memory cells, it could take decades for this reservoir of virus to be eliminated, even if all new rounds of infection were blocked.

Clinical Features of HIV Disease

A vast amount of information has accumulated about the epidemiology and clinical course of HIV infection. As antiretroviral drug therapy is improving, many of the clinical manifestations are changing. In the following section, we will describe the classic features of HIV infection and refer to the changing pictures when relevant.

Transmission of HIV and Epidemiology of AIDS

HIV is transmitted from one individual to another by three major routes:

- ***Sexual contact is the most frequent mode of transmission,*** either between heterosexual couples (the most frequent mode of transmission in Africa and Asia) or between men having sex with men. In sub-Saharan Africa, where the infection rate is the highest in the world (estimated to be several thousand new cases every day), more than half the infected individuals are women.
- ***Mother-to-child transmission*** of HIV accounts for the majority of pediatric cases of AIDS. This type of transmission occurs most frequently in utero or during childbirth, although transmission through breast milk is also possible.
- ***Inoculation of a recipient with infected blood or blood products*** is also a frequent mode of HIV transmission. Needles shared by intravenous drug users account for most cases of this form of transmission. HIV can remain infectious in a used infected needle for 6 weeks in temperate climates. With the advent of routine laboratory screening, transfusion of blood or blood products in a clinical setting accounts for a negligible proportion of HIV infections.

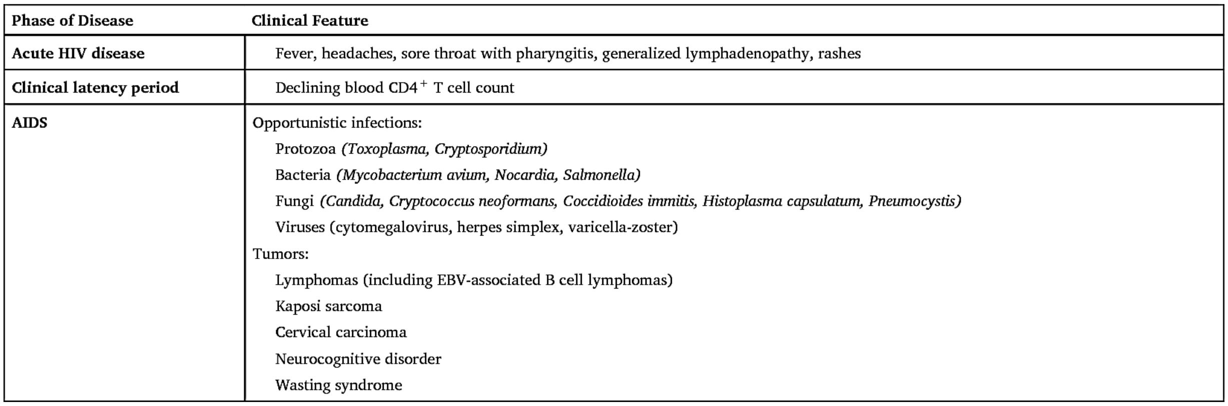
Clinical Course of HIV Infection

The course of HIV disease can be followed by measuring the amount of virus in the patient's plasma and by the blood CD4$^+$ T cell count (see Fig. 21.8). Three phases of the disease are recognized.

- The **acute phase**, also called acute HIV syndrome, is the period of viremia characterized by nonspecific symptoms of infection. It develops in 50% to 70% of infected adults typically 3 to 6 weeks after infection. There is a spike of plasma virus and a modest reduction in $CD4^+$ T cell counts, but the number of blood $CD4^+$ T cells often returns to normal. In many patients, however, the infection is occult and there are no symptoms.
- The **chronic phase of clinical latency** may last for many years. During this time, the virus is contained within lymphoid tissues and the loss of $CD4^+$ T cells is corrected by replenishment from progenitors. Patients are asymptomatic or suffer from minor infections. Within 2 to 6 months after infection, the concentration of plasma virus stabilizes at a particular set-point, which differs among patients. The level of the viral set-point and the number of blood $CD4^+$ T cells are clinically useful predictors of the progression of disease. As the disease progresses, patients become susceptible to other infections, and immune responses to these infections may stimulate HIV production and accelerate the destruction of lymphoid tissues. As discussed earlier, HIV gene transcription can be enhanced by stimuli that activate infected T cells, such as antigens and various cytokines. Cytokines, such as TNF, which are produced during the innate immune response to microbial infections, are particularly effective in boosting HIV production. Thus, as the immune system attempts to eradicate other microbes, it brings about its own destruction by HIV, a tragic example of what has been called subversion from within.
- ***HIV disease progresses to the final phase, called AIDS, when the blood $CD4^+$ T cell count drops below 200 cells/mm^3.*** HIV viremia may climb dramatically as viral replication accelerates unchecked in reservoirs other than T cells. Patients with AIDS suffer from combinations of

opportunistic infections, neoplasms, cachexia (HIV wasting syndrome), kidney failure (HIV nephropathy), and CNS disease (AIDS encephalopathy, now called HIV-associated neurocognitive disorder, or HAND) (Table 21.7). Because $CD4^+$ helper T cells are essential for both cell-mediated and humoral immune responses to various microbes, the loss of these lymphocytes is the main reason that patients with AIDS become susceptible to many different types of infections. Furthermore, many of the tumors that arise in patients with AIDS have a viral cause, and their prevalence in the setting of AIDS reflects an inability of the HIV-infected patient to mount an effective immune response against oncogenic viruses. Cachexia is often seen in patients with chronic inflammatory diseases and may result from effects of inflammatory cytokines (such as TNF) on appetite and metabolism. The CNS disease in AIDS may be due to neuronal damage by the virus or by shed viral proteins, such as gp120 and Tat, as well as the effects of cytokines elaborated by infected microglial cells.

Table 21.7



Phase of Disease	**Clinical Feature**
Acute HIV disease	Fever, headaches, sore throat with pharyngitis, generalized lymphadenopathy, rashes
Clinical latency period	Declining blood $CD4^+$ T cell count
AIDS	Opportunistic infections: Protozoa *(Toxoplasma, Cryptosporidium)* Bacteria *(Mycobacterium avium, Nocardia, Salmonella)* Fungi *(Candida, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Pneumocystis)* Viruses (cytomegalovirus, herpes simplex, varicella-zoster) Tumors: Lymphomas (including EBV-associated B cell lymphomas) Kaposi sarcoma Cervical carcinoma Neurocognitive disorder Wasting syndrome

AIDS, Acquired immunodeficiency syndrome; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus.

Although this summary of the clinical course is true for the most severe cases, the rate of progression of the disease is highly variable, and some individuals are long-term nonprogressors. The possible mechanisms underlying long-term nonprogression are described later. Importantly, antiretroviral therapy, which is now increasingly available, has changed the course of the disease and greatly reduced the incidence of severe opportunistic infections (such as *Pneumocystis*) and tumors (such as Kaposi sarcoma).

Immune Responses to HIV

Innate Immunity to HIV and Host Restriction Factors

Host restriction factors inhibit viral infection, and many viral proteins have evolved to counter these restriction factors. Host restriction factors are best appreciated in the overall context of innate immune responses to HIV. HIV is sensed by a number of pattern recognition receptors, including TLRs and RIG-I. Two key sensors that recognize viral reverse transcription products early in infection are IFI16 (interferon inducible protein 16) and cGAS (cyclic GMP-AMP synthase), both discussed in Chapter 4. IFI16 can bind to HIV-derived cDNA and signals via the STING (stimulator of IFN genes) adaptor, the TBK1 protein kinase, and the IRF3 and IRF7 transcription factors. This signaling induces type I IFN production and the expression of host restriction factors such as APOBEC3, TRIM5α, SAMHD1, and tetherin, described next.

Tetherin is a host factor that prevents virion release in certain cell types. It prevents the pinching off of certain viruses, including HIV, and its inhibition of the budding process can be antagonized by an HIV protein called Vpu. Host cells incorporate certain restriction factors into the virus particle, including APOBEC3 (apolipoprotein B mRNA editing enzyme catalytic polypeptide like 3) proteins. This protein is a cytidine deaminase that interferes with viral replication in infected cells. The HIV Vif protein helps target APOBEC3 proteins for ubiquitination and proteasomal degradation and thus promotes viral replication. In infected cells, another important host restriction factor is TRIM5α, a member of the TRIM (tripartite motif) family of ubiquitin E3 ligases. TRIM5α interacts with HIV capsid proteins to cause premature uncoating of the virus and proteasomal degradation of the viral reverse transcriptase complex. It can also block nuclear translocation of the viral pre-integration complexes. SAMHD1 (SAM domain and HD domain 1) is a host enzyme that hydrolyzes and depletes intracellular deoxynucleoside triphosphates and thus prevent synthesis of viral DNA by reverse transcription. The HIV-2 virus strain produces a protein called Vpx that antagonizes the depleting activity of SAMHD1.

Many other innate immune responses against HIV have been described. These include production of antimicrobial peptides (defensins) and activation of NK cells, DCs (particularly plasmacytoid DCs producing type I IFNs), and the complement system.

Adaptive Immune Responses to HIV

HIV-specific humoral and cell-mediated immune responses develop after infection but generally provide limited protection. The early immune response to HIV infection is, in fact, similar to the response to other viruses and serves to clear most of the virus present in the blood and in circulating T cells. Nonetheless, it is clear that these immune responses fail to eradicate HIV, and the infection eventually overwhelms the immune system in most untreated individuals. Despite the poor effectiveness of antiviral immune responses, it is important to characterize them for three reasons. First, the immune responses may be detrimental to the host, for example, by stimulating the uptake of opsonized virus into uninfected cells by Fc receptor–mediated endocytosis. Second, antibodies against HIV are diagnostic markers of HIV infection that are widely used for screening purposes. Third, the design of effective vaccines for immunization against HIV requires knowledge of the types of immune responses that are most likely to be protective (the immunologic correlates of protection).

The initial adaptive immune response to HIV infection is characterized by expansion of CD8 $^{+}$ T cells specific for HIV peptides. As many as 10% or more of circulating CD8^{+} T cells may be specific for HIV gag and other viral proteins during acute infection. These CTLs control infection in the early phase (see Fig. 21.8) but ultimately prove ineffective because of the emergence of viral escape mutants (variants with mutated antigens). $CD4^+$ T cells also respond to the virus, and these $CD4^+$ T cells may contribute to viral control in a number of ways. An effective $CD4^+$ T cell response is required as a source of help for the generation of $CD8^+$ memory T cells, but $CD4^+$ T cells have also been shown to kill HIV-infected cells. The importance of CTL responses in HIV control is underscored by the evolution of the virus under immune pressure, resulting in viral isolates that have lost their original CTL epitopes. The evolution of the virus also results in the loss of epitopes recognized by $CD4^+$ T cells, indicating that both $CD8^+$ and $CD4^+$ cells contribute to host defense against the virus.

Antibody responses to a variety of HIV antigens are detectable within 6 to 9 weeks after infection. The most immunogenic HIV molecules that elicit antibody responses appear to be the envelope glycoproteins, and high titers of anti-gp120 and anti-gp41 antibodies are present in most HIV-infected individuals. Other anti-HIV antibodies found frequently in patients' sera include antibodies to p24, reverse transcriptase, and *gag* and *pol* products (see Fig. 20.8). The effect of these antibodies on the clinical course of HIV infection is uncertain. The early antibodies are generally not neutralizing and are thus poor inhibitors of viral infectivity or cytopathic effects. Neutralizing antibodies against gp120 develop 2 to 3 months after primary infection, but even these antibodies cannot cope with a virus that is able to rapidly change the most immunodominant epitopes of its envelope glycoproteins. Sequencing of antibody heavy- and light-chain genes from gp-140–specific B cells in subjects who have been infected with HIV-1 for a few years has revealed the presence of broadly neutralizing antibodies. Intriguingly, for unknown reasons, only approximately 10% to 15% of chronically infected individuals develop broadly neutralizing antibodies. These antibodies bind to a site on a viral protein that the virus cannot afford to mutate, such as the CD4 binding site of gp140. They are, therefore, effective in clearing the virus. A striking feature of all of these antibodies is that they have been selected after extensive somatic hypermutation, implying helper T cell–dependent antibody responses. The implication is that the starting naive HIV-specific B cell repertoire primarily consists of B cells whose antigen receptors bind weakly to certain antigenic epitopes, such as the CD4 binding site of gp140. Many rounds of somatic hypermutation and selection that may occur in a long-standing infection can eventually generate B cell populations that bind with high affinity to the original weakly recognized epitope. One of the goals of vaccination is to generate such high-affinity broadly neutralizing antibodies, but inducing the high rate of somatic hypermutation necessary to achieve this has proved a challenge, and this has not yet been achieved with any consistency.

Mechanisms of Immune Evasion by HIV

HIV is the prototype of an infectious pathogen that evades host defenses by destroying the immune system. We have earlier considered some mechanisms by which this virus evades host restriction factors and innate immunity. In addition, several features of HIV may help the virus to evade adaptive immunity.

HIV has an extremely high mutation rate because of error-prone reverse transcription, and in this way it may evade detection by antibodies or T cells generated in response to viral proteins. A region of the gp120 molecule, called the V3 loop, is one of the most antigenically variable parts of the virus; it differs even in HIV isolates taken from the same individual at different times. Many epitopes of the virus that could potentially serve as targets for broadly neutralizing antibodies are also shielded by bulky N-linked sugars that make up what is known as the HIV-glycan shield.

HIV-infected cells may evade CTLs through downregulation of class I MHC molecule expression. The HIV Nef protein inhibits expression of class I MHC molecules, mainly by promoting internalization of these molecules. Other mechanisms of inhibiting cell-mediated immunity have been demonstrated in some cases. As mentioned earlier, these include a preferential inhibition of Th1 cytokines, activation of regulatory T cells, and suppression of dendritic cell functions. The mechanisms of these actions of the virus and their pathogenic significance are not established.

Elite Controllers and Long-Term Nonprogressors: A Possible Role for Host Genes

Although most individuals infected with HIV eventually develop AIDS, approximately 1% of individuals who are infected do not develop clinical disease. Such individuals have high $CD4^+$ and $CD8^+$ T cell counts, do not require therapy, and may have persistent viremia but no disease for at least 10 to 15 years. On the basis of the degree of viremia, this group can be divided into two subsets: long-term nonprogressors have detectable viremia of approximately 5000 copies of HIV-1 RNA per milliliter of blood, and a much smaller subset of elite controllers maintain viral loads of approximately 50 copies or less of HIV-1 RNA per milliliter of blood. There is considerable interest in understanding the genetic basis of HIV control by examining these cohorts of individuals in detail. So far, a strong role for the MHC locus in protecting individuals and preventing progression has been suggested by genetic association studies. Specific HLA class I loci have been linked to the absence of disease progression. Some elite controllers generate strong $CD8^+$ T cell responses to highly conserved peptides in the virus so that the virus cannot mutate without losing infectivity. We have previously mentioned the importance of the inheritance of the CCR5 homozygous 32-bp deletion in protection from infection, and other genetic factors contributing to resistance are likely to be revealed in the coming years.

Treatment and Prevention of AIDS and Vaccine Development

Active research efforts have been aimed at developing drugs that interfere with the viral life cycle. Treatment of HIV infection and AIDS now typically involves the administration of three antiviral drugs, used in combination, that target viral molecules for which no human homologues exist. The first antiretroviral drugs to be widely used were nucleoside analogues that bind to viral reverse transcriptase and inhibit its activity. These drugs include deoxythymidine nucleoside analogues such as AZT (3′-azido-3′-deoxythymidine), deoxycytidine nucleoside analogues, and deoxyadenosine analogues. When these drugs are used alone, they are often effective in significantly reducing plasma HIV RNA levels for several months to years, but they usually do not halt progression of HIV-induced disease, largely because of the evolution of virus with mutated forms of reverse transcriptase that are resistant to the drugs. Nonnucleoside reverse transcriptase inhibitors directly bind to the enzyme and inhibit its function. Viral protease inhibitors have been developed that block the processing of precursor proteins into mature viral capsid and core proteins. When these protease inhibitors are used alone, mutant viruses resistant to their effects emerge. However, protease inhibitors are now a common component of a three-drug therapeutic regimen with two different reverse transcriptase inhibitors. This triple-drug therapy, commonly referred to as highly active antiretroviral therapy (HAART) or antiretroviral therapy (ART), has proved to be effective in reducing plasma viral RNA to undetectable levels in most treated patients for years. An integrase inhibitor is now also available for antiviral therapy. Entry inhibitors, which prevent viral entry by targeting either CD4 or CCR5 on the host cell are another novel category of therapeutics. Fusion inhibitors are drugs that target gp41 and prevent fusion of the viral envelope with the host cell plasma membrane. Although antiretroviral therapy has reduced viral titers to below detection for up to 10 years in some patients, it is unlikely that such treatment can eliminate the virus from all reservoirs (especially long-lived infected cells), and resistance to the drugs may ultimately develop. Other formidable problems associated with these new drug therapies, which will impair their effective use in many parts of the world, include high expense and significant adverse effects. Furthermore, in some patients the virus evolves to become resistant to the drugs being used. This problem is often managed by sequencing the viral genome to identify mutations that may make the virus drug-resistant, and change the drug regimen accordingly. Inconsistent use of these drugs by patients is also a major problem. Pre-exposure prophylaxis (PrEP) using a single pill that contains two mechanistically different reverse transcriptase inhibitors is designed to protect individuals who do not have HIV but who are at high risk for getting the disease. Although PrEP has been effective in some contexts, its inconsistent use has proved a major problem and the rollout of PrEP has so far not blunted the epidemic in those parts of the world where the need is greatest.

A proportion of patients receiving ART experience an aberrant manifestation of immune reconstitution, namely overexuberant inflammation that may be triggered by the recognition by the immune system of preexisting pathogens. It usually accompanies the restoration of $CD4^+$ T cell counts and a decline in viral load. This clinical phenomenon is called the immune reconstitution inflammatory syndrome (IRIS).

The individual infections experienced by patients with AIDS are treated with the appropriate prophylaxis, antibiotics, and supportive measures. More aggressive antibiotic therapy is often required than for similar infections in less compromised hosts.

Although antiretroviral therapy has led to longer survival rates after HIV infection, chronic HIV infection is associated with an increased risk for non–AIDS-related morbidity and mortality than seen in age-matched uninfected individuals. In these patients there is a well-documented increase in cardiovascular disease, liver disorders, disorders of neurocognition, kidney disease, and some cancers. Chronic HIV is now recognized as a chronic inflammatory disease with manifestations that may be linked to local or systemic inflammation. This syndrome is the focus of many ongoing studies.

Efforts at prevention of HIV infection are extremely important and potentially effective in controlling the HIV epidemic. In the United States, the routine screening of blood products for evidence of donor HIV infection has already reduced the risk for this mode of transmission to negligible levels. Various public health measures to increase condom use and to reduce the use of contaminated needles by intravenous drug users are now widespread. Perhaps the most effective efforts at prevention are campaigns to increase public awareness of HIV. Clinical trials have shown that administration of antiretroviral drugs to pregnant mothers is effective at preventing infection of the newborns. Prophylactic use of these drugs in high-risk patients also reduces the rate of infection, as mentioned.

The development of an effective vaccine against HIV is a priority for biomedical research institutions worldwide. The task has been complicated by the ability of the virus to mutate and vary many of its immunogenic antigens. It is likely that an effective vaccine will have to stimulate both humoral and cell-mediated responses to viral antigens. To achieve this goal, several approaches are being tried for HIV vaccine development. Much of the preliminary work has involved infection of macaques with simian immunodeficiency virus (SIV), and effective vaccines against SIV have been developed. This success is encouraging because SIV is molecularly closely related to HIV and causes a disease in macaques that is similar to AIDS in humans. Various live virus vaccines have been tested in the hope that they will induce strong CTL responses. Such vaccines include nonvirulent recombinant hybrid viruses composed of part SIV and part HIV sequences or viruses that have been attenuated by deletions in one or more parts of the viral genome, such as the *nef* gene. One concern with live virus vaccines is their potential to cause disease if they are not completely attenuated and possibly to recombine with wild-type HIV to produce a pathogenic variant. Another approach that avoids this safety concern but retains efficacy in inducing CTL-mediated immunity is the use of live recombinant non-HIV viral vectors carrying HIV genes. Many DNA vaccines also have been studied; these vaccines are composed of combinations of structural and regulatory genes of SIV or HIV packaged in mammalian DNA expression vectors. T cell vaccines focusing on conserved regions of the viral proteome represent an interesting approach that is being undertaken and is currently in its early stages.

Most current vaccination attempts involve recombinant subunit vaccines with HIV trimers and are aimed at generating broadly neutralizing antibodies to gp140. A broadly neutralizing antibody is one that can prevent the infectivity of a large number of HIV strains, and such an antibody would therefore have to recognize some part of gp140, such as the CD4 binding site for instance, which is highly conserved. Human trials are currently under way with recombinant gp140 trimers in adenoviral vectors, based on adenoviral strains with low human seroprevalence. An interesting and inventive approach has involved the identification of the rearranged Ig genes that encode broadly neutralizing monoclonal human antibodies derived from individual clonal B cells isolated from a subset of chronic progressors. These antibodies may arise too late to be useful for the patients who generate them, but they provide lessons about how such antibodies evolve in vivo and have proved to be important for current vaccine generation attempts. It is possible to identify naive B cells that have rearranged Ig heavy and light chain genes that can somatically mutate after activation and give rise to these specific protective broadly neutralizing antibodies. These advances have led to the rational design of gp140 immunogens, based on structural biology, that can potentially activate B cells in a way that facilitates the stepwise evolution of somatically mutated broadly neutralizing antibodies. These promising approaches have surprisingly proved successful in mice engineered to express human Ig genes and may enter the clinical realm one day.

Other approaches for protection against HIV include the use of passive immunity with mixtures of broadly neutralizing antibodies, derived as described earlier, that have been recombinantly generated for clinical use. Early clinical studies have been promising. Vectored immunoprophylaxis is a form of immunity in which a protein that can mediate immune protection is synthesized in the host typically after injection of specific DNA into the skeletal muscle. In one approach that is currently being attempted in a clinical trial, the Ig heavy and light chain genes that encode a broadly neutralizing antibody against HIV have been cloned into an adenovirus-associated virus vector, and this DNA has been injected into the muscle of volunteers. An alternative approach that has worked well in simian models is the expression in the host of a fusion gene made up of CD4-Ig linked to a small CCR5-mimetic sulfopeptide. This fusion protein effectively prevents the entry of SIV into $CD4^+$ T cells. It is possible that if a vaccine against HIV does not prove successful, vectored immunoprophylaxis approaches may be used to staunch the spread of HIV.

Summary

- Immunodeficiency diseases are caused by congenital or acquired defects in lymphocytes, phagocytes, and other mediators of adaptive and innate immunity. These diseases are associated with an increased susceptibility to infection, the nature and severity of which depend largely on which component of the immune system is abnormal and the extent of the abnormality.
- Disorders of innate immunity include defects in microbial killing by phagocytes (e.g., chronic granulomatous disease or Chédiak-Higashi syndrome), leukocyte migration and adhesion (e.g., leukocyte adhesion deficiency), Toll-like receptor signaling, and complement.
- Severe combined immunodeficiencies include defects in lymphocyte development that affect both T and B cells and are caused by defective cytokine signaling, abnormal purine metabolism, defective V(D)J recombination, and mutations that affect T cell maturation.
- Antibody immunodeficiencies include diseases caused by defective B cell maturation or activation and defects in T-B

cell collaboration (X-linked hyper-IgM syndrome).

- T cell immunodeficiencies include diseases in which the expression of major histocompatibility complex molecules is defective, T cell signaling disorders, and rare diseases involving cytotoxic T lymphocyte and natural killer cell functions.
- Treatment of congenital immunodeficiencies involves transfusions of antibodies, stem cell transplantation, or enzyme replacement. Gene therapy may offer improved treatments in the future.
- Acquired immunodeficiencies are caused by infections, malnutrition, disseminated cancer, and immunosuppressive therapy for transplant rejection or autoimmune diseases.
- AIDS is a severe immunodeficiency caused by infection with HIV. This retrovirus infects $CD4^+$ T lymphocytes, macrophages, and dendritic cells and causes progressive dysfunction of the immune system. Most of the immunodeficiency in AIDS can be attributed to the depletion of $CD4^+$ T cells.
- HIV enters cells by binding to both the CD4 molecule and a coreceptor of the chemokine receptor family. After it is inside the cell, the viral genome is reverse-transcribed into DNA and incorporated into the cellular genome. Viral gene transcription and viral reproduction are stimulated by signals that normally activate the host cell. Production of virus is accompanied by death of infected cells.
- The acute phase of infection is characterized by death of activated and memory $CD4^+$ T cells in mucosal tissues and dissemination of the virus to lymph nodes. In the subsequent latent phase, there is low-level virus replication in lymphoid tissues and slow, progressive loss of T cells. Persistent activation of T cells promotes their death, leading to rapid loss and immune deficiency in the chronic phase of the infection.

- $CD4^+$ T cell depletion in HIV-infected individuals is largely due to direct cytopathic effects of the virus.
- Several reservoirs of HIV exist in infected individuals, including short-lived activated $CD4^+$ T cells, longer-lived macrophages, follicular helper T cells and very long-lived, latently infected memory T cells especially in mucosal sites.
- HIV-induced depletion of $CD4^+$ T cells results in increased susceptibility to infection by a number of opportunistic microorganisms. In addition, HIV-infected patients have an increased incidence of tumors, particularly Kaposi sarcoma and EBV-associated B cell lymphomas, and encephalopathy. The incidence of these complications has been greatly reduced by antiretroviral therapy.
- HIV has a high mutation rate, which allows the virus to evade host immune responses and become resistant to drug therapies. Genetic variability also poses a problem for the design of an effective vaccine against HIV. HIV infection can be treated by a combination of inhibitors of viral enzymes.

Selected Readings

∗Indicates publications of historical interest, generally reporting the discovery of a phenomenon or process that was later shown to be of fundamental importance in the immune system. Many (but not all) of these discoveries led to Nobel Prizes for the discoverer(s). The nature of the discovery is summarized briefly in each reference.

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Glossary

A

αβ T cell receptor (αβ TCR) The most common form of TCR, expressed on $CD4^+$ and $CD8^+$ T cells. The αβ TCR recognizes peptide antigen bound to an MCH molecule. Both α and β chains contain highly variable (V) regions that together form the antigen-binding site as well as constant (C) regions. TCR V and C regions are structurally homologous to the V and C regions of Ig molecules.

ABO blood group antigens Carbohydrate antigens attached mainly to cell surface proteins or lipids that are present on many cell types, including red blood cells and endothelial cells. These antigens differ among individuals, depending on inherited alleles encoding the enzymes required for synthesis of the carbohydrate antigens. The ABO antigens act as alloantigens that are responsible for blood transfusion reactions and hyperacute rejection of allografts.

Acquired immunodeficiency A deficiency in the immune system that is acquired after birth, often because of bone marrow damage or infection (e.g., AIDS) and that is not related to a genetic defect. Synonymous with **secondary immunodeficiency.**

Acquired immunodeficiency syndrome (AIDS) A disease caused by human immunodeficiency virus (HIV) infection that is characterized by depletion of $CD4^+$ T cells, leading to a profound immune deficiency. Clinically, AIDS includes opportunistic infections, malignant tumors, wasting, and encephalopathy.

Activation-induced cell death (AICD) Apoptosis of activated lymphocytes, generally used for T cells.

Activation-induced (cytidine) deaminase (AID) An enzyme expressed in B cells that catalyzes the conversion of cytosine into uracil in DNA, which is a step required for somatic hypermutation and affinity maturation of antibodies and for Ig class switching.

Activation protein 1 (AP-1) A family of DNA-binding transcription factors composed of dimers of two proteins that bind to one another through a shared structural motif called a leucine zipper. The best-characterized AP-1 factor is composed of the proteins FOS and JUN. AP-1 is involved in transcriptional regulation of many different genes that are important in the immune system, such as cytokine genes.

Active immunity The form of adaptive immunity that is induced by exposure to a foreign antigen and activation of lymphocytes and in which the immunized individual plays an active role in responding to the antigen. This type contrasts with passive immunity, in which an individual receives antibodies or lymphocytes from another individual who was previously actively immunized.

Acute-phase proteins Proteins, mostly synthesized in the liver in response to inflammatory cytokines such as IL-1, IL-6, and TNF, whose plasma concentrations increase shortly after infection. Examples include C-reactive protein, complement proteins, fibrinogen, and serum amyloid A protein. Acute-phase proteins play various roles in the innate immune response to microbes. Also called acute-phase reactants.

Acute-phase response The increase in plasma concentrations of several proteins, called acute-phase proteins (reactants), that occurs as part of the early innate immune response to infections.

Acute rejection A form of graft rejection involving vascular and parenchymal injury mediated by T cells, macrophages, and antibodies that usually first occurs days or weeks after transplantation but may occur later if pharmacologic immunosuppression becomes inadequate.

Adaptive immunity The form of immunity that is mediated by lymphocytes and stimulated by exposure to infectious agents. In contrast to innate immunity, adaptive immunity is characterized by exquisite specificity for distinct antigens and by long-term and specific memory, manifest as more rapid and vigorous responses on repeated exposure to the same antigen. Adaptive immunity is also called specific immunity or acquired immunity.

Adaptor protein A protein involved in intracellular signal transduction pathways that serves as a bridge molecule or scaffold for the recruitment of other signaling molecules. During lymphocyte antigen receptor or cytokine receptor signaling, adaptor molecules may be phosphorylated on tyrosine residues to enable them to bind other proteins containing SRC homology 2 (SH2) domains. Adaptor molecules involved in T cell activation include LAT, SLP76, and GRB2.

Addressin An adhesion molecule expressed on endothelial cells in different anatomic sites that directs organ-specific lymphocyte homing. Mucosal addressin cell adhesion molecule 1 (MAdCAM-1) is an example of an addressin expressed in Peyer's patches in the intestinal wall that binds to the integrin $\alpha_4\beta_7$ on gut-homing T cells.

Adhesion molecule A cell surface molecule whose function is to promote adhesive interactions with other cells or the extracellular matrix. Leukocytes express various types of adhesion molecules, such as selectins, integrins, and members of the Ig superfamily, and these molecules play crucial roles in cell migration and cellular activation in innate and adaptive immune responses.

Adjuvant A substance, distinct from antigen, that enhances T and B cell activation mainly by promoting the accumulation and activation of APCs at the site of antigen exposure. Adjuvants, which are routinely used in clinical vaccines and experimental animal immunizations, stimulate expression of T cell–activating costimulators and cytokines by APCs and may also prolong the expression of peptide-MHC complexes on the surface of APCs.

Adoptive transfer The process of transferring cells from one individual into another or back into the same individual after in vitro expansion and activation. Adoptive transfer is used in research to define the role of a particular cell population (e.g., T cells) in an immune response. Clinically, adoptive transfer of tumor-specific T lymphocytes and tumor antigen-presenting dendritic cells is used in cancer therapy.

Affinity The strength of the binding between a single binding site of a molecule (e.g., an antibody) and a ligand (e.g., an antigen). The affinity of a molecule X for a ligand Y is represented by the dissociation constant (K_d), which is the concentration of Y that is required to occupy the combining sites of half the X molecules present in a solution. A smaller K_d indicates a stronger or higher affinity interaction, because a lower concentration of ligand is needed to occupy the sites.

Affinity maturation The process that leads to increased affinity of antibodies for a particular antigen as a T cell–dependent antibody response progresses. Affinity maturation takes place in germinal centers of lymphoid tissues and is the result of somatic mutation of Ig genes, followed by selective survival of the B cells producing the highest affinity antibodies.

AKT A serine/threonine protein kinase involved in regulating many cellular processes, including growth, cell cycle, metabolism, genome stability, and apoptosis. In immune cells, AKT is activated by signals from antigen receptors, cytokine receptors, and GPCR ligands, leading to downstream signaling events that include mTOR activation. It is also known as protein kinase B (PKB).

Allele One of different variations of the same gene present at a particular chromosomal locus, each differing in nucleotide sequence, which often results in a different amino acid sequence of the encoded protein. An individual who is heterozygous at a locus has two different alleles, each on a different member of a pair of chromosomes, one inherited from the mother and one from the father. If a particular gene in a population has different alleles, the gene or locus is said to be polymorphic. MHC genes have many alleles (i.e., they are highly polymorphic).

Allelic exclusion The exclusive expression of only one of two inherited alleles encoding Ig heavy and light chains and TCR β chains. Allelic exclusion occurs when the protein product of one productively recombined antigen receptor locus on one chromosome blocks rearrangement and expression of the corresponding locus on the other chromosome. This ensures that most lymphocytes will express a single antigen receptor and that all antigen receptors expressed by one clone of lymphocytes will have the identical specificity. Because the TCR α chain locus does not show allelic exclusion, some T cells express two different TCRs.

Allergen An antigen that elicits an immediate hypersensitivity (allergic) reaction. Allergens are proteins or chemicals bound to proteins that induce IgE antibody responses in atopic individuals.

Allergy A disorder caused by immediate hypersensitivity reactions, often named according to the type of antigen (allergen) that elicits the disease, such as food allergy, bee sting allergy, and penicillin allergy. All of these conditions are the result of IgE production stimulated by IL-4- and IL-13–producing helper T cells, followed by allergen and IgE-dependent mast cell activation.

Alloantibody An antibody specific for an alloantigen (i.e., an antigen present in some individuals of a species but not in others).

Alloantigen A cell or tissue antigen that is present in some individuals of a species but not in others and that is recognized as foreign on an allograft. Alloantigens are usually products of polymorphic genes.

Alloantiserum The alloantibody-containing serum of an individual who has previously been exposed to one or more alloantigens.

Allograft An organ or tissue graft from a donor who is of the same species but genetically nonidentical to the recipient.

Alloreactive Reactive to alloantigens; describes T cells or antibodies from one individual that will recognize antigens on cells or tissues of another genetically nonidentical individual.

Allotype The property of a group of antibody molecules defined by their sharing of a particular antigenic determinant found on the antibodies of some individuals but not others. Allotype is also often used synonymously with allotope.

Alternative macrophage activation Macrophage activation by IL-4 and IL-13 leading to an antiinflammatory and tissue-reparative phenotype, in contrast to classical macrophage activation induced by IFN-γ and TLR ligands.

Alternative pathway of complement activation An antibody-independent pathway of activation of the complement system that occurs when the spontaneously generated C3b fragment of the C3 protein binds to microbial cell surfaces. The alternative pathway is a component of the innate immune system and mediates inflammatory responses to infection as well as direct lysis of microbes. The alternative pathway, as well as the classical and lectin pathways, terminate with formation of the membrane attack complex.

Anaphylatoxins The C5a, C4a, and C3a complement fragments that are generated during complement activation. The anaphylatoxins bind specific cell surface receptors and promote acute inflammation by stimulating neutrophil chemotaxis and activating mast cells.

Anaphylaxis A severe form of immediate hypersensitivity in which there is systemic mast cell or basophil activation, and the released mediators cause bronchial constriction, tissue edema, and cardiovascular collapse.

Anchor residues The amino acid residues of a peptide that fit into pockets in the floor of the peptide-binding cleft of an MHC molecule. These amino acids bind to complementary amino acids in the MHC molecule and therefore serve to anchor the peptide in the cleft of the MHC molecule.

Anergy A state of unresponsiveness to antigenic stimulation. Lymphocyte anergy (also called clonal anergy) is the failure of clones of T or B cells to react to antigen and is a mechanism for maintaining immunologic tolerance to self. Clinically, anergy describes the lack of T cell–dependent cutaneous delayed-type hypersensitivity reactions to common antigens.

Angiogenesis New blood vessel formation regulated by a variety of protein factors elaborated by cells of the innate and adaptive immune systems and often accompanying chronic inflammation.

Antibody A type of glycoprotein molecule, also called immunoglobulin (Ig), produced only by B lymphocytes and plasma cells derived from B cells, which binds antigens, often with a high degree of specificity and affinity. Membrane-bound antibodies serve as antigen receptors that initiate B cell activation. Secreted antibodies perform various effector functions, including neutralizing antigens, activating complement, and promoting leukocyte-dependent destruction of microbes. The basic structural unit of an antibody is composed of two identical heavy chains and two identical light chains. The N-terminal variable regions of the heavy and light chains form the antigen-binding sites, whereas the C-terminal constant regions of the heavy chains of secreted antibodies interact with other molecules in the immune system. Every individual has millions of different antibodies, each with a unique antigen-binding site.

Antibody-dependent cell-mediated cytotoxicity (ADCC) A process by which NK cells are targeted to IgG-coated cells, resulting in lysis of the antibody-coated cells. A specific receptor for the constant region of IgG, called FcγRIII (CD16), is expressed on the NK cell membrane and mediates binding to the IgG.

Antibody feedback The downregulation of antibody production by secreted IgG antibodies that occurs when antigen-antibody complexes simultaneously engage B cell membrane Ig and one type of Fcγ receptor (FcγRIIb). Under these conditions, the cytoplasmic tail of FcγRIIb transduces inhibitory signals inside the B cell.

Antibody repertoire The collection of different antibody specificities expressed in an individual.

Antibody-secreting cell A B lymphocyte that has undergone differentiation and produces the secreted form of Ig. Antibody-secreting cells are generated from naive B cells in response to antigen and other stimuli and reside in the spleen, lymph nodes, and bone marrow. Often used synonymously with plasma cells.

Antigen A molecule that binds to an antibody or a TCR. Antigens that bind to antibodies include all classes of molecules. Most TCRs bind only peptide fragments of proteins complexed with MHC molecules.

Antigen presentation The display of peptides bound by MHC molecules on the surface of an antigen presenting cell that permits specific recognition by TCRs and activation of T cells.

Antigen-presenting cell (APC) A cell that displays peptide fragments of protein antigens, in association with MHC molecules, on its surface and activates antigen-specific T cells. In addition to displaying peptide-MHC complexes, APCs also express costimulatory molecules to optimally activate T lymphocytes.

Antigen processing The intracellular conversion of protein antigens derived from the extracellular space or the cytosol into peptides and loading of these peptides onto MHC molecules for display to T lymphocytes.

Antigenic variation The process by which antigens expressed by microbes may change by various genetic mechanisms, and therefore allow the microbe to evade immune responses. One example of antigenic variation is the change in influenza virus surface proteins hemagglutinin and neuraminidase that necessitates the use of new vaccines each year.

Antimicrobial peptide One of a group of peptides with both positively charged and hydrophobic amino acid residues, which insert into and disrupt the function of the outer membranes of bacteria and some viruses. Antimicrobial peptides, including cathelicidins, defensins, and regIII peptides, are produced by epithelial barrier cells and leukocytes and serve as innate immune effector molecules.

Antiretroviral therapy (ART) Combination chemotherapy for HIV infection, usually consisting of nucleoside reverse transcriptase inhibitors and either a viral protease inhibitor or a nonnucleoside reverse transcriptase inhibitor. ART can reduce plasma virus titers to below detectable levels and slow the progression of HIV disease. Also called highly active antiretroviral therapy (HAART).

Antiserum Serum from an individual previously immunized with an antigen that contains antibody specific for that antigen.

Apoptosis A process of cell death characterized by activation of intracellular caspases, DNA cleavage, nuclear condensation and fragmentation, and plasma membrane blebbing that leads to phagocytosis of cell fragments without inducing an inflammatory response. Apoptosis is important in development of lymphocytes, return to homeostasis after an immune response to an infection, maintenance of tolerance to self antigens, and killing of infected cells by cytotoxic T lymphocytes and natural killer cells. Apoptosis is one form of programmed cell death; others include autophagy and pyroptosis.

Arthus reaction A localized form of experimental immune complex–mediated cutaneous vasculitis induced by injection of an antigen subcutaneously into a previously immunized animal or into an animal that has been given intravenous antibody specific for the antigen. Circulating antibodies bind to the injected antigen and form immune complexes that are deposited in the walls of small arteries at the injection site and give rise to a local vasculitis with necrosis.

Asthma An inflammatory disease characterized by intermittent and reversible airway obstruction, due to bronchial smooth muscle cell hypertrophy and hyperreactivity, chronic bronchial inflammation with eosinophils, and excess bronchial mucus production. Most cases are caused by Th2, IgE, and mast cell–mediated allergic reactions (atopic asthma), but a minority are caused by nonimmune stimuli such as exercise, exposure to cold, or certain drugs (nonatopic asthma).

Atopy The propensity of an individual to mount Th2 responses and produce IgE antibodies in response to various environmental antigens and to develop strong immediate hypersensitivity (allergic) responses. People who have allergies to environmental antigens, such as pollen or house dust, are said to be atopic.

Autoantibody An antibody specific for a self antigen. Autoantibodies can cause damage to cells and tissues and are produced in excess in autoimmune diseases, such as systemic lupus erythematosus.

Autocrine factor A molecule that acts on the same cell that produces the factor. For example, IL-2 is an autocrine T cell growth factor that stimulates proliferation of the T cell that produces it.

Autoimmune disease A disease caused by a breakdown of self-tolerance such that the adaptive immune system responds to self antigens and mediates cell and tissue damage. Autoimmune diseases can be caused by immune attack against one organ or tissue (e.g., multiple sclerosis, thyroiditis, or type 1 diabetes) or against multiple and systemically distributed antigens (e.g., systemic lupus erythematosus).

Autoimmune regulator (AIRE) A protein that functions to stimulate expression of peripheral tissue protein antigens in medullary thymic epithelial cells, which is required for tolerance to those antigens. Mutations in the gene encoding AIRE in humans and mice impair expression of tissue antigens in the thymus and cause an autoimmune disease because of failure to delete T cells or to generate regulatory T cells specific for these antigens.

Autoimmunity The state of adaptive immune system responsiveness to self antigens that occurs when mechanisms of self-tolerance fail.

Autologous graft A tissue or organ graft in which the donor and recipient are the same individual. Autologous bone marrow and skin grafts are performed in clinical medicine.

Autophagy The physiological process by which a cell degrades its own components by lysosomal digestion. Autophagy plays a role in innate immune defense against infections, and polymorphisms of genes that regulate autophagy are linked to risk for some autoimmune diseases.

Avidity The overall strength of interaction between two molecules, such as an antibody and antigen. Avidity depends on both the affinity and the valency of interactions. Therefore, the avidity of a pentameric IgM antibody, with 10 antigen-binding sites, for a multivalent antigen may be greater than the avidity of a dimeric IgG molecule for the same antigen. Avidity can also be used to describe the strength of cell-cell interactions, which are mediated by many binding interactions between cell surface molecules.

B

B lymphocyte The only cell type capable of producing antibody molecules and therefore the mediator of humoral immune responses. B lymphocytes, or B cells, develop in the bone marrow, and mature B cells are found mainly in lymphoid follicles in secondary lymphoid tissues, in bone marrow, and in low numbers in the circulation.

B-1 lymphocytes A subset of B lymphocytes that develop earlier during ontogeny than do follicular B cells, express a limited repertoire of V genes with little junctional diversity, and secrete IgM antibodies in response to T-independent antigens. B-1 cells are located mainly in mucosal tissues.

Bare lymphocyte syndrome An immunodeficiency disease characterized by a lack of class II MHC molecule expression that leads to defects in antigen presentation and cell-mediated immunity. The disease is caused by mutations in genes encoding factors that regulate class II MHC gene transcription.

Basophil A type of bone marrow–derived circulating granulocyte with structural and functional similarities to mast cells that has granules containing many of the same inflammatory mediators as mast cells and expresses a high-affinity Fc receptor for IgE. Basophils that are recruited into tissue sites where antigen is present may contribute to immediate hypersensitivity reactions.

BCL-6 A transcriptional repressor that is required for germinal center B cell development and for Tfh cell development.

BCL-2 family proteins A family of partially homologous cytoplasmic and mitochondrial membrane proteins that regulate apoptosis by influencing mitochondrial outer membrane permeability. Members of this family can be pro-apoptotic (e.g., BAX, BAD, and BAK) or anti-apoptotic (e.g., BCL-2 and BCL-X_L).

B cell receptor (BCR) The cell surface antigen receptor on B lymphocytes, which is a membrane bound Ig molecule.

B cell receptor complex (BCR complex) A multiprotein complex expressed on the surface of B lymphocytes that recognizes antigen and transduces activating signals into the cell. The BCR complex includes membrane Ig, which is responsible for binding antigen, and Igα and Igβ proteins, which initiate signaling events.

BLIMP1 A transcriptional repressor that is required for plasma cell generation.

Bispecific T cell engagers (BiTEs) Recombinant antibody-derived molecules consisting of two fused Ig single-chain variable fragments (scFvs), one specific for the CD3 molecule on T cells and the other for a tumor antigen. They bring together T cells with tumor cells, and activate the T cell to kill the tumor cell.

Bone marrow The tissue within the central cavity of bone that is the site of generation of all circulating blood cells in adults, including immature lymphocytes, and the site of B cell maturation.

Bone marrow transplantation *See* **Hematopoietic stem cell transplantation.**

Bruton tyrosine kinase (BTK) A TEC family tyrosine kinase that is essential for B cell maturation. Mutations in the gene encoding BTK cause X-linked agammaglobulinemia, a disease characterized by failure of B cells to mature beyond the pre-B cell stage.

Burkitt's lymphoma A malignant B cell tumor that is diagnosed by histologic features but almost always carries a reciprocal chromosomal translocation involving *Ig* gene loci and the *MYC* gene on chromosome 8. Many cases of Burkitt's lymphoma in Africa are associated with Epstein-Barr virus infection.

C

C (constant region) gene segments The DNA sequences in the *Ig* and *TCR* gene loci that encode the nonvariable portions of Ig heavy and light chains and TCR α, β, γ, and δ chains.

C1 A plasma complement system protein composed of several polypeptide chains that initiates the classical pathway of complement activation by attaching to the Fc portions of IgG or IgM antibody that has bound antigen.

C1 inhibitor (C1 INH) A plasma protease inhibitor that blocks esterase functions of the C1r and C1s components of complement C1, which initiate the classical pathway of complement activation. C1 INH also blocks proteases in fibrinolytic, coagulation, and kinin pathways. C1 INH is a serine protease inhibitor (serpin), which mimics the structure of the substrates of the proteases it inhibits. A genetic deficiency in C1 INH causes hereditary angioedema, a disease characterized mainly by deregulated bradykinin activity.

C2 A classical complement pathway protein that is proteolytically cleaved by activated C1 to generate C2a, which forms part of the classical pathway C3 convertase.

C3 The central and most abundant complement system protein; it is involved in the classical, alternative and lectin complement pathways. C3 is proteolytically cleaved during complement activation to generate a C3b fragment, which covalently attaches to cell or microbial surfaces where it acts as an opsonin; and a C3a fragment, sometimes called an anaphylatoxin, that has various proinflammatory activities.

C3 convertase A multiprotein enzyme complex generated by the early steps of classical, alternative, and lectin pathways of complement activation. C3 convertase cleaves C3, which gives rise to two proteolytic products, C3a and C3b.

C4 A classical complement pathway protein that is proteolytically cleaved by activated C1 to generate C4b, which forms part of the classical pathway C3 convertase, and a C4a fragment, sometimes called an anaphylatoxin, which has various proinflammatory activities.

C5 A protein that is cleaved by C5 convertases in all complement pathways, generating a C5b fragment, which initiates formation of the membrane attack complex, and a C5a fragment, sometimes called an anaphylatoxin, which has various proinflammatory activities.

C5 convertase A multiprotein enzyme complex generated by C3b binding to C3 convertase. C5 convertase cleaves C5 and initiates the late steps of complement activation leading to formation of the membrane attack complex and lysis of cells.

Calcineurin A cytoplasmic serine/threonine phosphatase that dephosphorylates the transcription factor NFAT, thereby allowing NFAT to enter the nucleus. Calcineurin is activated by calcium signals generated through TCR signaling in response to antigen recognition, and the immunosuppressive drugs cyclosporine and tacrolimus work by blocking calcineurin activity.

Carcinoembryonic antigen (CEA, CD66) A highly glycosylated membrane protein; increased expression of CEA in many carcinomas of the colon, pancreas, stomach, and breast results in a rise in blood levels. The level of blood CEA is used to monitor the persistence or recurrence of metastatic carcinoma after treatment.

Caspases Intracellular proteases with cysteines in their active sites that cleave substrates at the C-terminal sides of aspartic acid residues. Most are components of enzymatic cascades that cause apoptotic death of cells, but caspase-1, which is part of the inflammasome, drives inflammation by processing inactive precursor forms of the cytokines IL-1 and IL-18 into their active forms.

Cathelicidins Peptides produced by neutrophils and various barrier epithelia that serve various functions in innate immunity, including direct toxicity to microorganisms, activation of leukocytes, and neutralization of lipopolysaccharide. Along with defensins, cathelicidins are often called antimicrobial peptides (AMPs).

Cathepsins Thiol and aspartyl proteases with broad substrate specificities, which are abundant in the endosomes in APCs and play an important role in generating peptide fragments that bind to class II MHC molecules from exogenous protein antigens.

CD molecules Cell surface molecules expressed on various cell types in the immune system that are designated by the "cluster of differentiation" or CD number. *See* Appendix I for a list of CD molecules.

Cell-mediated immunity (CMI) The form of adaptive immunity that is mediated by T lymphocytes and serves as the defense mechanism against various types of microbes that are taken up by phagocytes or infect nonphagocytic cells. Cell-mediated immune responses include CD4^{+} T cell–mediated activation of phagocytes and CD8^{+} CTL–mediated killing of infected cells.

Central tolerance A form of self-tolerance induced in primary (generative or central) lymphoid organs as a consequence of immature self-reactive lymphocytes recognizing self antigens and subsequently leading to their death (clonal deletion), change in specificity (receptor editing in B cells), or differentiation into regulatory T cells. Central tolerance prevents the emergence of lymphocytes with high-affinity receptors for the self antigens that are expressed in the bone marrow or thymus.

Centroblasts Rapidly proliferating B cells in the dark zone of germinal centers of secondary lymphoid tissues, which give rise to thousands of progeny, express activation-induced deaminase (AID), and undergo somatic mutation of their *V* genes. Centroblasts become the centrocytes of the light zone of germinal centers.

Centrocytes B cells in the light zone of germinal centers of secondary lymphoid organs, which are the progeny of proliferating centroblasts of the dark zone. Centrocytes that express high-affinity Ig are selected to survive and undergo isotype switching and further differentiation into long-lived plasma cells and memory B cells.

Checkpoint blockade A form of cancer immunotherapy in which blocking antibodies specific for T cell inhibitory molecules, including PD-1, PD-L1, and CTLA-4, are administered to cancer patients to boost antitumor T cell responses, also called immune checkpoint blockade. This approach has been successful in effectively treating several kinds of widely metastatic cancers that are unresponsive to other therapies.

Chédiak-Higashi syndrome A rare autosomal recessive immunodeficiency disease caused by a defect in the cytoplasmic granules of various cell types that affects the lysosomes of neutrophils and macrophages as well as the granules of CTLs and NK cells. Patients show reduced resistance to infection with pyogenic bacteria.

Chemokine receptors Cell surface receptors for chemokines that transduce signals stimulating the migration of leukocytes. There are at least 19 different mammalian chemokine receptors, each of which binds a different set of chemokines; all are members of the seven-transmembrane α-helical, G protein–coupled receptor family.

Chemokines A large family of structurally homologous low-molecular-weight cytokines that stimulate leukocyte chemotaxis, regulate the migration of leukocytes from the blood to tissues by activating leukocyte integrins, and maintain the spatial organization of different subsets of lymphocytes and APCs within lymphoid organs.

Chemotaxis Movement of a cell directed by a chemical concentration gradient. Leukocyte chemotaxis within various tissues is often directed by gradients of low-molecular-weight cytokines called chemokines, by the lipid sphingosine 1-phosphate, or by the bacterial peptide N-formylmethionyl-leucyl-phenylalanine.

Chimeric antigen receptor (CAR) Genetically engineered receptors with tumor antigen–specific binding sites encoded by recombinant Ig variable genes and cytoplasmic tails containing signaling domains of both the TCR and costimulatory receptors. When T cells are engineered to express chimeric antigen receptors, these cells can recognize and kill cells that the extracellular domain recognizes. Adoptive transfer of CAR-expressing T cells has been used successfully for the treatment of some types of hematologic cancers.

Chromosomal translocation A chromosomal abnormality in which a segment of one chromosome is transferred to another. Many malignant diseases of lymphocytes are associated with chromosomal translocations involving an Ig or TCR locus and a chromosomal segment containing a cellular oncogene.

Chronic granulomatous disease A rare inherited immunodeficiency disease caused by mutations in genes encoding components of the phagocyte oxidase enzyme complex that is needed for microbial killing by polymorphonuclear leukocytes and macrophages. The disease is characterized by recurrent intracellular bacterial and fungal infections, often accompanied by chronic cell-mediated immune responses and the formation of granulomas.

Chronic rejection A form of allograft rejection characterized by fibrosis with loss of normal organ structures occurring during a prolonged period. In many cases, the major pathologic event in chronic rejection is graft arterial occlusion caused by proliferation of intimal smooth muscle cells, which is called graft arteriosclerosis.

Citrullinated proteins Proteins in which arginine residues have been post-translationally converted to citrulline. Auto-antibodies specific for citrullinated proteins are found in patients with rheumatoid arthritis.

c-KIT ligand (stem cell factor) A protein required for hematopoiesis, early steps in T cell development in the thymus, and mast cell development. c-KIT ligand is produced in membrane-bound and soluble forms by stromal cells in the bone marrow and thymus, and it binds to the c-KIT tyrosine kinase membrane receptor on pluripotent stem cells.

Class I major histocompatibility complex (MHC) molecule One of two forms of polymorphic heterodimeric membrane proteins that bind and display peptide fragments of protein antigens on the surface of APCs for recognition by T lymphocytes. Class I MHC molecules usually display peptides derived from proteins in the cytosol of the cell, for recognition by CD8$^+$ T cells.

Class II–associated invariant chain peptide (CLIP) A peptide remnant of the invariant chain that sits in the class II MHC peptide-binding cleft and is removed by action of the HLA-DM molecule before the cleft becomes accessible to peptides produced by lysosomal proteolysis of extracellular protein antigens.

Class II major histocompatibility complex (MHC) molecule One of two major classes of polymorphic heterodimeric membrane proteins that bind and display peptide fragments of protein antigens on the surface of APCs for recognition by T lymphocytes. Class II MHC molecules usually display peptides derived from extracellular proteins that are internalized into phagocytic or endocytic vesicles, for recognition by CD4$^+$ T cells.

Classical macrophage activation Macrophage activation by IFN-γ, Th1 cells, and TLR ligands, leading to a proinflammatory and microbicidal phenotype. Classically activated macrophages are also called M1 macrophages.

Classical pathway of complement activation The complement pathway that is an effector arm of the humoral immunity, generating inflammatory mediators, opsonins for phagocytosis of antigens, and lytic complexes that destroy cells. The classical pathway is initiated by binding of antigen-antibody complexes to the C1 molecule, leading to proteolytic cleavage of C4 and C2 proteins to generate the classical pathway C3 convertase. The classical pathway, as well as the alternative and lectin pathways, terminate with formation of the membrane attack complex.

Clonal anergy A state of antigen unresponsiveness of a clone of T lymphocytes experimentally induced by recognition of antigen in the absence of additional costimulatory signals required for functional activation. Clonal anergy is considered a model for one mechanism of tolerance to self antigens and may be applicable to B lymphocytes as well.

Clonal deletion A mechanism of lymphocyte tolerance in which an immature T cell in the thymus or an immature B cell in the bone marrow undergoes apoptotic death as a consequence of recognizing a self antigen.

Clonal expansion The approximately 1000- to 100,000-fold increase in number of lymphocytes specific for an antigen that results from antigen stimulation and proliferation of T and B cells. Clonal expansion occurs in lymphoid tissues and is required to generate enough antigen-specific effector T lymphocytes and plasma cells from rare naive precursors to eradicate infections.

Clonal ignorance A form of lymphocyte unresponsiveness in which self antigens are ignored by the immune system even though lymphocytes specific for those antigens remain viable and functional.

Clonal selection hypothesis A fundamental tenet of the immune system (no longer a hypothesis) stating that every individual possesses numerous clonally derived lymphocytes, each clone having arisen from a single precursor, expresses one antigen receptor, and is capable of recognizing and responding to a distinct antigenic determinant. When an antigen enters, it selects a specific preexisting clone and activates it.

Clone A group of cells, all derived from a single common precursor, that maintain many of the genotypic and phenotypic features shared by the cell of origin. In adaptive immunity, all members of a clone of lymphocytes share the same clonally unique recombined *Ig* or *TCR* genes, although the rearranged *Ig V* genes of different cells within a clone of B cells may vary in sequence due to somatic hypermutation that occurs after VDJ recombination.

Coinhibition The inhibition of T cell activation mediated by molecules on APCs or other cells that bind to receptors on T cells, causing blockade of TCR and costimulatory signals in the T cell. Examples include PD-L1 on APCs or tumor cells binding to PD-1 on T cells, which generates inhibitory signals that block TCR and costimulatory signals in the T cell, or CTLA-4 on a T cell binding to B7-1 and B7-2 on an APC, preventing costimulation by B7-1 and B7-2 binding to CD28 on the T cell. Blockade of coinhibitory molecules is widely used as a therapeutic strategy to enhance antitumor immunity **(checkpoint blockade).**

Collectins A family of proteins, including mannose-binding lectin, that is characterized by a collagen-like domain and a lectin (i.e., carbohydrate-binding) domain. Collectins play a role in the innate immune system by acting as microbial pattern recognition receptors, and they may activate the complement system by binding to C1q.

Colony-stimulating factors (CSFs) Cytokines that promote the expansion and differentiation of bone marrow progenitor cells. CSFs are essential for the maturation of red blood cells, granulocytes, monocytes, and lymphocytes. Examples of CSFs include granulocyte-monocyte colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and IL-3.

Combinatorial diversity The diversity of Ig and TCR specificities generated by the use of many different combinations of different variable, diversity, and joining segments during somatic recombination of DNA in the Ig and TCR loci in developing B and T cells. Combinatorial diversity is one mechanism that works together with junctional diversity for the generation of large numbers of different antigen receptor genes from a limited number of gene segments.

Common variable immunodeficiency disease (CVID) One of a group of heterogeneous disorders characterized by reduced circulating antibody, impaired antibody responses to infection and vaccines, increased incidence of infections, typically with *Haemophilus influenzae* and *Streptococcus pneumoniae,* various autoimmune manifestations, and a high incidence of lymphomas. Most cases are sporadic, but up to 25% of patients have a family history, some of which are a result of monogenic mutations.

Complement A system of plasma and cell surface proteins that interact with one another and with other molecules of the immune system to generate important effectors of innate and adaptive immune responses. The classical, alternative, and lectin pathways of the complement system are activated by antigen-antibody complexes, microbial surfaces, and plasma lectins binding to microbes, respectively, and consist of a cascade of proteolytic enzymes that generate inflammatory mediators and opsonins. All three pathways lead to the formation of a common terminal cell lytic complex that is inserted in cell membranes.

Complement receptor type 1 (CR1, or CD35) A receptor for the C3b and C4b fragments of complement. Phagocytes use CR1 to mediate internalization of C3b- or C4b-coated particles. CR1 on erythrocytes serves in the clearance of immune complexes from the circulation. CR1 is also a regulator of complement activation.

Complement receptor type 2 (CR2, or CD21) A receptor expressed on B cells and follicular dendritic cells that binds proteolytic fragments of the C3 complement protein, including C3d, C3dg, and iC3b. CR2 functions to stimulate humoral immune responses by enhancing B cell activation by antigen and by promoting the trapping of antigen-antibody complexes in germinal centers. CR2 is also a receptor for Epstein-Barr virus.

Complement receptor type 3 (CR3, or CD11bCD18) An integrin expressed mainly on neutrophils and macrophages, which binds to a peptide fragment of C3 called iC3b, which is deposited on microbes as a result of complement pathway activation. CR3 mediates the phagocytosis of iC3b coated microbes.

Complementarity-determining regions (CDRs) Short segments of Ig and TCR proteins that contain most of the sequence differences between different antibodies or TCRs and make contact with antigen; also called **hypervariable regions.** Three CDRs are present in the variable domain of each antigen receptor polypeptide chain, and six CDRs are present in an intact Ig or TCR molecule. These hypervariable segments assume loop structures that together form a surface complementary to the three-dimensional structure of the bound antigen.

Congenic mouse strains Inbred mouse strains that are identical to one another at every genetic locus except the one for which they are selected to differ; such strains are created by repetitive back-crossbreeding and selection for a particular trait. Congenic strains that differ from one another only at a particular MHC allele have been useful in defining the function of MHC molecules.

Congenital immunodeficiency *See* **Primary immunodeficency.**

Constant (C) region The portion of Ig or TCR polypeptide chains that is not involved in antigen binding and is not highly variable between clones of B or T cells.

Contact sensitivity A reaction to certain chemical agents leading to T cell–mediated delayed-type hypersensitivity reactions on skin contact. Substances that elicit contact sensitivity, including nickel ions, urushiols in poison ivy, and many therapeutic drugs, bind to and modify self proteins on the surfaces of APCs, which are then recognized by $CD4^+$ or $CD8^+$ T cells.

Coreceptor A lymphocyte surface receptor that binds to an antigen at the same time that membrane Ig or TCR binds the antigen and delivers signals required for optimal lymphocyte activation. CD4 and CD8 are T cell coreceptors that bind nonpolymorphic parts of an MHC molecule concurrently with the TCR binding to polymorphic MHC residues and the displayed peptide. CR2 is a coreceptor on B cells that binds to complement-coated antigens at the same time that membrane Ig binds an epitope of the antigen.

Costimulator A molecule expressed on the surface of APCs in response to innate immune stimuli, which provides a stimulus (the “second signal”), in addition to antigen, required for the activation of naive T cells. The best defined costimulators are the B7 molecules (CD80 and CD86) on APCs that bind to the CD28 receptor on T cells. Sometimes the receptor (CD28) is called a costimulator.

COVID-19 A disease caused by the highly infectious coronavirus SARS-CoV-2, characterized mainly by respiratory system symptoms, often with pneumonia, but also affecting various other organ systems. An exuberant inflammatory response to the virus causes irreversible and often lethal lung injury in a minority of patients. The COVID-19 pandemic of 2020-2021 has led to the infection of millions of people worldwide, and over 2 million people have died of the disease.

CpG nucleotides Unmethylated cytidine-guanine sequences found mainly in microbial DNA that stimulate innate immune responses. CpG oligonucleotides are recognized by TLR9, and they are used as adjuvants.

C-reactive protein (CRP) A member of the pentraxin family of plasma proteins involved in innate immune responses to bacterial infections. CRP binds to the capsule of pneumococcal bacteria. CRP also binds to C1q and may thereby activate complement or act as an opsonin by interacting with phagocyte C1q receptors. CRP is an acute-phase protein, and increased plasma concentration of CRP is a clinically used marker of inflammation.

Cross-matching A screening test performed to minimize the chance of adverse transfusion reactions or graft rejection, in which a patient in need of a blood transfusion or organ allograft is tested for the presence of preformed antibodies against donor cell surface antigens (usually blood group antigens or MHC antigens). The test involves mixing the recipient serum with leukocytes or red blood cells from potential donors and analyzing for agglutination or complement-dependent lysis of the cells.

Cross-presentation A mechanism by which a dendritic cell (DC) activates (or primes) a naive CD8$^+$ CTL specific for the antigens of a third cell (e.g., a virus-infected or tumor cell). Cross-presentation occurs, for example, when protein antigens from an infected cell are ingested by a DC and the microbial antigens are processed and presented in association with class I MHC molecules, unlike the general rule for phagocytosed antigens, which are presented in association with class II MHC molecules. The DC also provides costimulation for the T cells. Also called **cross-priming.**

CTLA-4 An Ig superfamily protein expressed on the surface of activated effector T cells and Treg that binds B7-1 and B7-2 with high affinity and plays an essential role in inhibiting T cell responses. CTLA-4 is essential for Treg function and T cell tolerance to self antigens.

CTLA-4-Ig A recombinant fusion protein composed of the extracellular B7-binding portion of CTLA-4 linked to an IgG Fc region. CTLA-4 Ig a competitive inhibitor of B7 binding to CD28 and thereby blocks T cell costimulation. It is used as a drug to treat transplant rejection and autoimmune diseases. The IgG Fc enhances the duration the drug persists in the circulation. Note that CTLA-4-Ig is not anti-CTLA-4 antibody.

C-type lectin A member of a large family of calcium-dependent carbohydrate-binding proteins, many of which play important roles in innate and adaptive immunity. For example, soluble C-type lectins bind to microbial carbohydrate structures and mediate phagocytosis or complement activation (e.g., mannose-binding lectin, dectins, collectins, ficolins).

Cutaneous immune system The components of the innate and adaptive immune system found in the skin that function together in a specialized way to detect and respond to pathogens on or in the skin and to maintain homeostasis with commensal microbes. Components of the cutaneous immune system include keratinocytes, Langerhans cells, dermal dendritic cells, intraepithelial lymphocytes, and dermal lymphocytes.

Cyclic GMP-AMP synthase A cytosolic DNA sensor of the innate immune system that generates cyclic GMP-AMP as a second messenger and uses the STING adaptor to induce type I IFN synthesis.

Cyclosporine A calcineurin inhibitor widely used as an immunosuppressive drug to prevent allograft rejection by blocking T cell activation. Cyclosporine (also called cyclosporin A) binds to a cytosolic protein called cyclophilin, and cyclosporine-cyclophilin complexes bind to and inhibit calcineurin, thereby inhibiting activation and nuclear translocation of the transcription factor NFAT.

Cytokine release syndrome A life-threatening systemic inflammatory condition, sometime referred to as cytokine storm, due to secretion of massive amounts of inflammatory cytokines (including IFN-γ, TNF, and IL-6) by immune cells. Cytokine release syndrome has been associated with gram negative septicemia, CAR-T cell therapy of cancers, and infections with SARS CoV-2.

Cytokines Proteins that are produced and secreted by many different cell types, and mediate inflammatory and immune reactions. Cytokines are principal mediators of communication between cells of the immune system (*see* Appendix II).

Cytosolic DNA sensors (CDSs) Molecules that detect microbial double-stranded DNA in the cytosol and activate signaling pathways that initiate antimicrobial responses, including type I IFN production and autophagy.

Cytotoxic (or cytolytic) T lymphocyte (CTL) A type of T lymphocyte whose major effector function is to recognize and kill host cells infected with viruses or other intracellular microbes as well as tumor cells. CTLs usually express CD8 and recognize microbial peptides displayed by class I MHC molecules. CTL killing of infected cells involves delivery of the contents of cytoplasmic granules into the cytosol of infected cells, leading to apoptotic death.

D

Damage-associated molecular patterns (DAMPs) Endogenous molecules that are produced by or released from damaged and dying cells that bind to pattern recognition receptors and stimulate innate immune responses. Examples include high-mobility group box 1 (HMGB1) protein, extracellular ATP, and uric acid crystals.

Death receptors Plasma membrane receptors expressed on various cell types that, on ligand binding, transduce signals that lead to recruitment of the FAS-associated protein with death domain (FADD) adaptor protein, which activates caspase-8, leading to apoptotic cell death. All death receptors, including FAS, TRAIL, and TNFR, belong to the TNF receptor superfamily.

Dectins Pattern recognition receptors expressed on dendritic cells that recognize fungal cell wall carbohydrates and induce signaling events that promote inflammation and enhance adaptive immune responses.

Defensins Cysteine-rich peptides produced by epithelial barrier cells in the skin, gut, lung, and other tissues and in neutrophil granules that act as broad-spectrum antibiotics to kill a wide variety of bacteria and fungi. The synthesis of defensins is increased in response to stimulation of innate immune system receptors such as Toll-like receptors and inflammatory cytokines such as IL-1 and TNF. Cathelicidins and defensins are two types of antimicrobial peptides (AMPs).

Delayed-type hypersensitivity (DTH) An immune reaction in which T cell–dependent macrophage activation and inflammation cause tissue injury. A DTH reaction to the subcutaneous injection of antigen is often used as an assay for cell-mediated immunity (e.g., the purified protein derivative skin test for immunity to *Mycobacterium tuberculosis*).

Dendritic cells Bone marrow–derived cells found in epithelial barriers, the stroma of most organs, and lymphoid tissues that are morphologically characterized by thin membranous projections. Many subsets of dendritic cells exist with diverse functions. Classical (conventional) dendritic cells (cDCs) function as innate sentinel cells and become APCs for naive T lymphocytes on activation, and they are important for initiation of adaptive immune responses to protein antigen. Plasmacytoid dendritic cells (pDCs) produce abundant type I IFNs in response to exposure to viruses. Monocyte-derived dendritic cells (MoDCs) are derived from blood monocytes during inflammatory reactions.

Desensitization A method of treating immediate hypersensitivity disorders (allergies) that involves repetitive administration of low doses of an antigen to which individuals are allergic. This process often prevents severe allergic reactions on subsequent environmental exposure to the antigen, but the mechanisms are not well understood.

Determinant The specific portion of a macromolecular antigen to which an antibody or TCR binds. In the case of a protein antigen recognized by a T cell, the determinant is the peptide portion that is displayed by an MHC molecule for recognition by the TCR. Synonymous with **epitope.**

Diacylglycerol (DAG) A signaling molecule generated by phospholipase C (PLCγ)-mediated hydrolysis of the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) during antigen activation of lymphocytes, as well as activation of various other immune cells. The main function of DAG is to activate an enzyme called protein kinase C that participates in the generation of active transcription factors.

DiGeorge syndrome A selective T cell deficiency caused by a congenital malformation that results in defective development of the thymus, parathyroid glands, and other structures that arise from the third and fourth pharyngeal pouches.

Direct antigen presentation (or direct allorecognition) Presentation of cell surface allogeneic MHC molecules by graft APCs to a graft recipient's T cells that leads to activation of the alloreactive T cells. In direct recognition of an allogeneic MHC molecule, a TCR that was selected to recognize a self MHC molecule plus foreign peptide cross-reacts with the intact allogeneic MHC molecule plus a random peptide. Direct presentation is partly responsible for strong T cell responses to allografts.

Diversity The existence of a large number of lymphocytes with different antigenic specificities in any individual. Diversity is a fundamental property of the adaptive immune system and is the result of variability in the structures of the antigen-binding sites of lymphocyte receptors for antigens (antibodies and TCRs).

Diversity (D) segments Short coding sequences between the variable (V) and constant (C) gene segments in the Ig heavy chain and TCR β and γ loci that together with J segments are somatically recombined with V segments during lymphocyte development. The resulting recombined VDJ DNA codes for the carboxy-terminal ends of antigen receptor V regions, including the third hypervariable (CDR) regions. Random use of D segments contributes to the diversity of the antigen receptor repertoire.

DNA vaccine A vaccine composed of a bacterial plasmid containing a complementary DNA encoding a protein antigen. DNA vaccines presumably work because professional APCs are transfected in vivo by the plasmid and express immunogenic peptides that elicit specific responses. Furthermore, the plasmid DNA contains CpG nucleotides that act as potent adjuvants.

Double-negative thymocyte A subset of developing T cells in the thymus (thymocytes) that express neither CD4 nor CD8. Most double-negative thymocytes are at an early developmental stage and do not express antigen receptors. They will later express both CD4 and CD8 during the intermediate double-positive stage before further maturation to single-positive T cells expressing only CD4 or CD8.

Double-positive thymocyte A subset of developing T cells in the thymus (thymocytes) that express both CD4 and CD8 and are at an intermediate developmental stage. Double-positive thymocytes also express TCRs and are subject to selection processes, and they mature to single-positive T cells expressing only CD4 or CD8.

E

Ectoparasites Parasites that live on the surface of an animal, such as ticks and mites. Both the innate and adaptive immune systems may play a role in protection against ectoparasites, often by destroying the larval stages of these organisms.

Effector cells The cells that perform effector functions during an immune response, such as secreting cytokines (e.g., helper T cells), killing microbes (e.g., macrophages), killing microbe-infected host cells (e.g., CTLs), or secreting antibodies (e.g., plasma cells).

Effector phase The phase of an immune response in which a foreign antigen is destroyed or inactivated. For example, in a humoral immune response, the effector phase may be characterized by antibody-dependent complement activation and phagocytosis of antibody- and complement-opsonized bacteria.

Endosome An intracellular membrane-bound vesicle into which extracellular proteins are internalized during antigen processing. Endosomes are formed by invagination of the plasma membrane, and they may mature into late endosomes and lysosomes, which have progressively lower pH and more hydrolytic enzymes. The proteolytic enzymes in endosomes degrade internalized proteins into peptides that bind to class II MHC molecules, and the endosomes containing these peptides fuse with Golgi-derived vesicles containing class II MHC molecules. (Endosomes are found in all cells and participate in internalization events that are not linked to antigen presentation.)

Endotoxin A component of the cell wall of gram-negative bacteria, also called **lipopolysaccharide (LPS),** that is released from dying bacteria and stimulates innate immune inflammatory responses by binding to TLR4 on many different cell types, including phagocytes, endothelial cells, dendritic cells, and barrier epithelial cells. Endotoxin contains both lipid components and carbohydrate (polysaccharide) moieties.

Enhancer A regulatory nucleotide sequence in a gene that is located either upstream or downstream of the promoter, binds transcription factors, and increases the activity of the promoter. In cells of the immune system, enhancers are responsible for integrating cell surface signals that lead to induced transcription of genes encoding many of the effector proteins of an immune response, such as cytokines.

Envelope glycoprotein (Env) A membrane glycoprotein encoded by a retrovirus that is expressed on the plasma membrane of infected cells and on the host cell–derived membrane coat of viral particles. Env proteins are often required for viral infectivity. The Env proteins of HIV include gp41 and gp120, which bind to CD4 and chemokine receptors, respectively, on human T cells and mediate fusion of the viral and T cell membranes.

Enzyme-linked immunosorbent assay (ELISA) A method of quantifying an antigen immobilized on a solid surface by use of a specific antibody with a covalently coupled enzyme. The amount of antibody that binds the antigen is proportional to the amount of antigen present and is determined by spectrophotometrically measuring the conversion of a clear substrate to a colored product by the coupled enzyme (*see* Appendix III).

Eosinophil A bone marrow–derived granulocyte that is abundant in the inflammatory infiltrates of immediate hypersensitivity late-phase reactions and contributes to many of the pathologic processes in allergic diseases. They are recognizable by their bright red cytosolic granules in standard Wright-stained blood smears and hematoxylin and eosin–stained tissue sections. Eosinophils are important in defense against extracellular parasites, including helminths.

Epitope The specific portion of a macromolecular antigen to which an antibody or TCR binds. In the case of a protein antigen recognized by a T cell, an epitope is the peptide portion that binds to an MHC molecule for recognition by the TCR. Synonymous with **determinant.**

Epitope spreading In autoimmunity, the development of immune responses to multiple epitopes as an autoimmune disease originally targeting one epitope progresses, likely caused by further breakdown in tolerance and release of additional tissue antigens due to the inflammatory process stimulated by the initial response.

Exhaustion *See* **T cell exhaustion**.

Epstein-Barr virus (EBV) A double-stranded DNA virus of the herpesvirus family that is the etiologic agent of infectious mononucleosis and is associated with some B cell malignant tumors and nasopharyngeal carcinoma. EBV infects B lymphocytes and some epithelial cells by specifically binding to complement receptor 2 (CR2, CD21).

Experimental autoimmune encephalomyelitis (EAE) An animal model of multiple sclerosis, an autoimmune demyelinating disease of the central nervous system. EAE is induced in rodents by immunization with components of the myelin sheath (e.g., myelin basic protein) of nerves, mixed with an adjuvant. The disease is mediated in large part by cytokine-secreting $CD4^+$ T cells specific for the myelin sheath proteins.

F

Fab (fragment, antigen-binding) A part of an antibody, first produced by proteolysis of IgG, that includes one complete light chain paired with one heavy chain fragment containing the variable domain and only the first constant domain. Fab fragments, which can be generated from all antibodies, retain the ability to bind an antigen but cannot interact with IgG Fc receptors on cells or with complement. Therefore, Fab preparations are used in research and therapeutic applications when antigen binding is desired without activation of effector functions. (The Fab′ fragment retains the hinge region of the heavy chain.)

F(ab′)$_2$ A part of an Ig molecule (first produced by proteolysis of IgG) that includes two complete light chains but only the variable domain, first constant domain, and hinge region of the two heavy chains. F(ab′)$_2$ fragments retain the entire bivalent antigen-binding region of an intact Ig molecule but cannot bind complement or Fc receptors. They are used in research and therapeutic applications when antigen binding is desired without antibody effector functions.

FAS (CD95) A death receptor of the TNF receptor family that is expressed on the surface of T cells and many other cell types and initiates a signaling cascade leading to apoptotic death of the cell. The death pathway is initiated when FAS ligand expressed on activated T cells binds to FAS on the same or other cells. FAS-mediated killing of lymphocytes plays a role in the maintenance of self-tolerance. Mutations in the *FAS* gene cause systemic autoimmune lymphoproliferative syndrome [ALPS]). (*See* also **Death receptor**).

FAS ligand (CD95 ligand) A membrane protein that is a member of the TNF family of proteins expressed on activated T cells. FAS ligand binds to the death receptor FAS, thereby stimulating a signaling pathway leading to apoptotic cell death of the FAS-expressing cell. Mutations in the FAS ligand gene cause systemic autoimmune disease in mice.

Fc (fragment, crystalline) A region of an antibody molecule that can be isolated by proteolysis of IgG that contains only the disulfide-linked carboxy-terminal regions of the two heavy chains. The Fc region of Ig molecules mediates effector functions by binding to cell surface receptors or the C1q complement protein. (Fc fragments are so named because they tend to crystallize out of solution.)

Fc receptor A cell surface receptor specific for the carboxy-terminal constant region of an Ig molecule. Fc receptors are typically multichain protein complexes that include signaling components and Ig-binding components. Several types of Fc receptors exist, including those specific for different IgG isotypes, IgE, and IgA. Fc receptors mediate many of the cell-dependent effector functions of antibodies, including phagocytosis of antibody-bound antigens, antigen-induced activation of mast cells, and targeting and activation of NK cells.

FcεRI A high-affinity receptor for the carboxyl-terminal constant region of IgE molecules that is expressed on mast cells, basophils, and eosinophils. FcεRI molecules on mast cells are usually occupied by IgE, and antigen-induced cross-linking of these IgE-FcεRI complexes activates the mast cell and initiates immediate hypersensitivity reactions.

Fcγ receptor (FcγR) A cell surface receptor specific for the carboxy-terminal constant region of IgG molecules. There are several different types of Fcγ receptors, including a high-affinity FcγRI that mediates phagocytosis by macrophages and neutrophils, a low-affinity FcγRIIB that transduces inhibitory signals in B cells and myeloid cells, and a low-affinity FcγRIIIA that mediates targeting and activation of NK cells.

Fibroblastic reticular cell (FRC) Mesenchymally derived cells that drive formation of secondary lymphoid organs during embryonic development and contribute in multiple ways to the structure and functions of these organs.

Ficolins Hexameric innate immune system plasma proteins, containing collagen-like domains and fibrinogen-like carbohydrate-recognizing domains, that bind to cell wall components of gram-positive bacteria, opsonizing them and activating complement.

First-set rejection Allograft rejection in an individual who has not previously received a graft or otherwise been exposed to tissue alloantigens from the same donor. First-set rejection usually takes approximately 7 to 14 days to develop after graft transplantation.

Flow cytometry A method of analysis of the phenotype of cell populations requiring a specialized instrument (flow cytometer) that can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule to which a fluorescent probe binds, as well as the relative amount of the molecule expressed. Suspensions of cells are incubated with fluorescently labeled antibodies or other probes, and the amount of probe bound by each cell in the population is measured by passing the cells one at a time through a fluorimeter with a laser-generated incident beam. (*See* Appendix III.)

Fluorescence-activated cell sorter (FACS) An adaptation of the flow cytometer that is used for the purification of cells from a mixed population according to which and how much fluorescent probe the cells bind. Cells are first stained with fluorescently labeled probe, such as an antibody specific for a surface antigen of a cell population. The cells are then passed one at a time through a fluorimeter with a laser-generated incident beam and are deflected into different collection tubes by electromagnetic fields whose strength and direction are varied according to the measured intensity of the fluorescence signal.

Follicle *See* **Lymphoid follicle.**

Follicular dendritic cells (FDCs) Cells in lymphoid follicles of secondary lymphoid organs that express complement receptors and Fc receptors and have long cytoplasmic processes that form a meshwork. Follicular dendritic cells display antigens on their surface for B cell recognition and are involved in the activation and selection of B cells expressing high-affinity membrane Ig during the process of affinity maturation. They are nonhematopoietic cells (not of bone marrow origin).

Follicular helper T cell (Tfh cell) *See* **T follicular helper (Tfh) cells.**

***N*-Formylmethionine** An amino acid that initiates all bacterial proteins but not mammalian proteins (except those synthesized within mitochondria) and serves as a signal to the innate immune system of infection. Specific receptors for *N*-formylmethionine–containing peptides are expressed on neutrophils and mediate activation of the neutrophils.

FOXP3 A forkhead family transcription factor expressed by and required for the development and function of $CD4^+$ regulatory T cells. Mutations in the gene ending FOXP3 in mice and humans result in an absence of $CD25^+$ regulatory T cells and multisystem autoimmune disease called IPEX.

G

γδ T cell receptor (γδ TCR) A form of TCR that is distinct from the more common αβ TCR and is expressed on a subset of T cells found mostly in epithelial barrier tissues. Although the γδ TCR is structurally similar to the αβ TCR, the forms of antigen recognized by γδ TCRs are poorly understood; they do not recognize peptide complexes bound to polymorphic MHC molecules.

G protein–coupled receptor family A diverse family of receptors for hormones, lipid inflammatory mediators, and chemokines that use associated trimeric G proteins for intracellular signaling.

G proteins Proteins that bind guanyl nucleotides and catalyze the replacement of bound guanosine diphosphate (GDP) by guanosine triphosphate (GTP). G proteins with bound GTP can activate a variety of cellular enzymes in different signaling cascades. Trimeric GTP-binding proteins are associated with the cytoplasmic portions of many cell surface receptors, such as chemokine receptors. Other small soluble G proteins, such as RAS and RAC, are recruited into signaling pathways by adaptor proteins.

Gasdermin D A cytosolic protein that is proteolytically cleaved by caspase-1 and other caspases in macrophages to generate a pore-forming fragment that inserts into the plasma membrane, leading to an inflammatory form of cell death called pyroptosis.

GATA3 A transcription factor that plays an essential role in the differentiation of Th2 cells from naive T cells and the differentiation of ILC2s.

Generative lymphoid organ An organ in which lymphocytes develop from immature precursors. The bone marrow and thymus are the major generative lymphoid organs in which B cells and T cells develop, respectively. Generative lymphoid organs are also called **primary** or **central lymphoid organs.**

Germinal centers Specialized structures in lymphoid organs generated during T-dependent humoral immune responses, where extensive B cell proliferation, somatic mutation, affinity maturation, memory B cell generation, and induction of long-lived plasma cells take place. Germinal centers appear as lightly staining regions within a lymphoid follicle in spleen, lymph node, and mucosal lymphoid tissue.

Germline organization The inherited arrangement of variable, diversity, joining, and constant region gene segments of the antigen receptor loci in nonlymphoid cells or in immature lymphocytes. In developing B or T lymphocytes, the germline organization is modified by somatic recombination to form functional *Ig* or *TCR* genes.

Glomerulonephritis Inflammation of renal glomeruli, often initiated by immunopathologic mechanisms such as deposition of circulating antigen-antibody complexes in the glomerular basement membrane or binding of antibodies to antigens expressed in the glomerulus. The antibodies can activate complement and phagocytes, and the resulting inflammatory response can lead to renal failure.

Graft A tissue or organ that is removed from one site and placed in another site, usually in a different individual.

Graft arteriosclerosis Occlusion of graft arteries caused by proliferation of intimal smooth muscle cells. This process is occurs gradually over years after transplantation and is largely responsible for chronic rejection and failure of vascularized organ grafts. The mechanism is likely to be a chronic immune response to vessel wall alloantigens. Graft arteriosclerosis is also called accelerated arteriosclerosis.

Graft rejection A specific immune response to an organ or tissue graft that leads to inflammation, damage, and possibly graft failure.

Graft-versus-host disease A disease occurring in bone marrow transplant recipients that is caused by the reaction of mature T cells in the marrow graft with alloantigens on host cells. The disease most often affects the skin, liver, and intestines.

Granulocyte colony-stimulating factor (G-CSF) A cytokine made by activated T cells, macrophages, and endothelial cells at sites of infection that acts on progenitors in the bone marrow to increase the production of and mobilize neutrophils to replace those consumed in inflammatory reactions.

Granulocyte-monocyte colony-stimulating factor (GM-CSF) A cytokine made by activated T cells, macrophages, endothelial cells, and stromal fibroblasts that acts on bone marrow progenitors to increase the production of neutrophils and monocytes. GM-CSF is also a macrophage-activating factor and promotes the maturation of dendritic cells.

Granuloma A nodule of inflammatory tissue composed of clusters of activated macrophages and T lymphocytes, usually with associated fibrosis. Granulomatous inflammation is a form of chronic delayed-type hypersensitivity, often in response to persistent microbes, such as *Mycobacterium tuberculosis* and some fungi, or in response to particulate antigens that are not readily phagocytosed.

Granulysin A lipid-binding cationic peptide found in granules of CTLs and NK cells, which can damage cholesterol-poor membranes, typical of bacteria but not mammalian cells, and thereby can kill intracellular microbes.

Granzyme B A serine protease enzyme found in the granules of CTLs and NK cells that is released by exocytosis, enters target cells, and proteolytically cleaves and activates caspases, which in turn induce target cell apoptosis.

Gut-associated lymphoid tissue (GALT) Collections of lymphocytes and APCs within the mucosa of the gastrointestinal tract, where adaptive immune responses to intestinal microbial flora and ingested antigens are initiated (*see* also **Mucosa-associated lymphoid tissues**).

H

H-2 molecule A major MHC molecule in the mouse. The mouse MHC was originally called the H-2 locus.

Haplotype The set of MHC alleles inherited from one parent and therefore on one chromosome.

Hapten A small chemical that can bind to an antibody but must be attached to a macromolecule (carrier) to stimulate an adaptive immune response specific for that chemical. For example, immunization with dinitrophenol (DNP) alone will not stimulate an anti-DNP antibody response, but immunization with a protein with covalently attached DNP hapten will.

Heavy-chain isotype (class) switching The process by which a B lymphocyte changes the isotype, or class, of the antibodies that it produces, from IgM to IgG, IgE, or IgA, without changing the antigen specificity of the antibody. Heavy-chain isotype switching is stimulated by cytokines and CD40 ligand expressed by T follicular helper cells and involves activation-induced cytidine deaminase (AID)–dependent recombination of B cell VDJ segments with downstream heavy-chain gene segments.

Helminth A parasitic worm. Helminthic infections often elicit Th2-dependent immune responses characterized by eosinophil-rich inflammatory infiltrates and IgE production.

Helper T (Th) cells The class of T lymphocytes whose main functions are to activate macrophages and to promote inflammation in cell-mediated immune responses and to promote B cell antibody production in humoral immune responses. These functions are mediated by secreted cytokines and by T cell CD40 ligand binding to macrophage or B cell CD40. Helper T cells express the CD4 molecule and recognize peptide antigens displayed by class II MHC molecules.

Hematopoiesis The development of mature blood cells, including erythrocytes, leukocytes, and platelets, from pluripotent stem cells in the bone marrow and fetal liver. Hematopoiesis is regulated by several different cytokine growth factors produced by bone marrow stromal cells, T cells, and other cell types.

Hematopoietic stem cell An undifferentiated stem cell in the fetal yolk sac or liver or in adult bone marrow that asymmetrically divides to give rise to additional stem cells and a cell that differentiates into one of multiple different lineages. A hematopoietic stem cell in the bone marrow will give rise to cells of the lymphoid, myeloid, and erythrocytic lineage.

Hematopoietic stem cell transplantation The transplantation of hematopoietic stem cells taken from the blood or bone marrow; it is performed clinically to treat hematopoietic or lymphopoietic disorders and malignant diseases and is also used in various immunologic experiments in animals.

High endothelial venules (HEVs) Specialized venules that are the sites of lymphocyte migration from the blood into the stroma of secondary lymphoid tissues. HEVs are lined by plump endothelial cells that protrude into the vessel lumen and express unique adhesion molecules involved in binding naive and central memory B and T cells.

Hinge region A region of Ig heavy chains between the first two constant domains that can assume multiple conformations, thereby imparting flexibility in the orientation of the two antigen-binding sites. Because of the hinge region, an antibody molecule can simultaneously bind two epitopes that are anywhere within a range of distances from one another.

Histamine A vasoactive amine stored in the granules of mast cells that is one of the important mediators of immediate hypersensitivity. Histamine binds to specific receptors in various tissues and causes increased vascular permeability and contraction of bronchial and intestinal smooth muscle.

HLA *See* **Human leukocyte antigens.**

HLA-DM A peptide exchange molecule that plays a critical role in the class II MHC pathway of antigen presentation. HLA-DM is found in the specialized endosomal compartment where antigens are processed and facilitates removal of the invariant chain–derived CLIP peptide and the binding of other peptides to class II MHC molecules. HLA-DM is encoded by a gene in the MHC and is structurally similar to class II MHC molecules, but it is not polymorphic.

Homeostasis In the adaptive immune system, the maintenance of a constant number and diverse repertoire of lymphocytes, despite the emergence of new lymphocytes and tremendous expansion of individual clones that may occur during responses to immunogenic antigens. Homeostasis is achieved by several regulated pathways of lymphocyte death and inactivation.

Homing receptor Adhesion molecules expressed on the surface of lymphocytes that are responsible for the different pathways of lymphocyte recirculation and tissue homing. Homing receptors bind to ligands (addressins) expressed on endothelial cells in particular vascular beds.

Human immunodeficiency virus (HIV) The etiologic agent of AIDS. HIV is a retrovirus that infects a variety of cell types, including CD4-expressing helper T cells, macrophages, and dendritic cells, and causes chronic progressive destruction of the immune system.

Human leukocyte antigens (HLA) MHC molecules expressed on the surface of human cells. Human MHC molecules include three types of class I MHC molecules (HLA-A, -B and -C) and three types of class II MHC molecules (HLA-DP, -DQ, and -DR). (*See* also **Major histocompatibility complex [MHC] molecule)**.

Humanized monoclonal antibody A monoclonal antibody encoded by a recombinant hybrid gene and composed of the antigen-binding sites from a murine monoclonal antibody and the constant region of a human antibody. Humanized antibodies are less likely than mouse monoclonal antibodies to induce an anti-antibody response in humans; they are used clinically in the treatment of inflammatory diseases, tumors, and transplant rejection. In current drug development, fully human recombinant monoclonal antibodies have largely replaced humanized mouse antibodies.

Humoral immunity The type of adaptive immune response mediated by antibodies produced by B lymphocytes. Humoral immunity is the principal adaptive immune defense mechanism against extracellular microbes and their toxins.

Hybridoma A cell line derived by fusion, or somatic cell hybridization, between a normal lymphocyte and an immortalized lymphocyte tumor line. B cell hybridomas created by fusion of normal B cells of defined antigen specificity with a myeloma cell line are used to produce monoclonal antibodies. T cell hybridomas created by fusion of a normal T cell of defined specificity with a T cell tumor line are used in research.

Hyperacute rejection A form of allograft or xenograft rejection that begins within minutes to hours after transplantation and is characterized by thrombotic occlusion of the graft vessels. Hyperacute rejection is mediated by preexisting antibodies in the host circulation that bind to donor endothelial alloantigens, such as blood group antigens or MHC molecules, and activate the complement system.

Hypersensitivity diseases Disorders caused by immune responses. Hypersensitivity diseases include autoimmune diseases, in which immune responses are directed against self antigens, and diseases that result from uncontrolled or excessive responses against foreign antigens, such as microbes and allergens. The tissue damage that occurs in hypersensitivity diseases is caused by the same effector mechanisms used by the immune system to protect against microbes.

Hypervariable region Short segments of approximately 10 amino acid residues within the variable regions of antibody or TCR proteins that form loop structures that contact antigen. Three hypervariable loops are present in each antibody heavy chain and light chain and in each TCR α and β chain. Most of the variability among different antibodies or TCRs is located within these loops (also called **complementarity determining region** [CDR]).

I

Idiotype The unique molecular structure of the antigen binding site of the antibodies or TCRs made by a single clone of B or T cells. The idiotype is determined by the sequence of amino acids in the hypervariable regions of antibody or TCR polypeptide chains.

Igα and Igβ Proteins that are required for surface expression and signaling functions of membrane Ig on B cells. Igα and Igβ pairs are disulfide linked to one another, and noncovalently associated with the cytoplasmic tail of membrane Ig, to form the BCR complex. The cytoplasmic domains of Igα and Igβ contain ITAMs that are involved in early signaling events during antigen-induced B cell activation.

IL-1 receptor antagonist (IL-1RA) A natural inhibitor of IL-1 produced by mononuclear phagocytes that is structurally homologous to IL-1 and binds to the same receptors but does not induce signaling. IL-1RA is used as a drug to treat autoinflammatory syndromes caused by dysregulated IL-1 production.

Immature B lymphocyte A membrane IgM$^+$, IgD$^-$ B cell, recently derived from marrow precursors, that does not proliferate or differentiate in response to antigens but rather may undergo apoptotic death or become functionally unresponsive. This property is important for the negative selection of B cells that are specific for self antigens present in the bone marrow.

Immediate hypersensitivity The type of immune reaction responsible for allergic diseases, which is dependent on antigen-mediated activation of IgE-coated tissue mast cells. The mast cells release mediators that cause increased vascular permeability, vasodilation, bronchial and visceral smooth muscle contraction, and local inflammation.

Immune complex A multimolecular complex of antibody molecules with bound antigen. Because each antibody molecule has a minimum of two antigen-binding sites and many antigens are multivalent, immune complexes can vary greatly in size. Immune complexes activate effector mechanisms of humoral immunity, such as the classical complement pathway and Fc receptor–mediated phagocyte activation. Deposition of circulating immune complexes in blood vessel walls or renal glomeruli can lead to inflammation and disease.

Immune complex disease Any inflammatory disease caused by the deposition of antigen-antibody complexes in blood vessel walls, resulting in local complement activation and inflammation. Immune complexes may form because of overproduction of antibodies against microbial antigens or as a result of autoantibody production in the setting of an autoimmune disease such as systemic lupus erythematosus. Immune complex deposition in the capillary basement membranes of renal glomeruli can cause glomerulonephritis and impair renal function. Systemic deposition of immune complexes in arterial walls can cause vasculitis, with thrombosis and ischemic damage to various organs.

Immune deviation The conversion of a T cell response associated with one set of cytokines, such as Th1 cytokines that stimulate inflammatory functions of macrophages, to a response associated with other cytokines, such as Th2 cytokines that activate eosinophils and antiinflammatory functions of macrophages.

Immune inflammation Inflammation that is a result of an adaptive immune response to antigen. The cellular infiltrate at the inflammatory site may include cells of the innate immune system, such as neutrophils and macrophages, that are recruited by the actions of T cell cytokines.

Immune response A collective and coordinated response to the introduction of foreign substances in an individual mediated by the cells and molecules of the immune system.

Immune response (Ir) genes Originally defined as genes in inbred strains of rodents that were inherited in a dominant Mendelian manner and that controlled the ability of the animals to make antibodies against simple synthetic polypeptides. Ir genes are the polymorphic genes that encode class II MHC molecules, which display peptides to T lymphocytes and are therefore required for T cell activation and helper T cell–dependent B cell (antibody) responses to protein antigens.

Immune surveillance The concept that a physiologic function of the immune system is to recognize and destroy clones of transformed cells before they grow into tumors and to kill tumors after they are formed. The term immune surveillance is sometimes used in a general sense to describe the function of T lymphocytes to detect and destroy any cell, not necessarily a tumor cell, that is expressing foreign (e.g., microbial) antigens.

Immune synapse The tight juxtaposition of membranes of a T cell and an APC, including the collection of membrane proteins that become organized at the point of juxtaposition, such as the TCR complex, CD4 or CD8, costimulatory receptors, and integrins on the T cell, which bind to peptide-MHC complexes, costimulators, and integrin ligands on the APC. The immune synapse is required for bidirectional functional responses between the T cell and APC, and enhances specific delivery of secreted products from the T cell to the APC, such as granule contents from a CTL to its target cell.

Immune system The molecules, cells, tissues, and organs that collectively function to provide immunity, or protection, against foreign organisms.

Immunity Protection against disease, usually infectious disease, mediated by the cells and tissues that are collectively called the immune system. In a broader sense, immunity refers to the ability to respond to foreign substances, including microbes and noninfectious molecules.

Immunoblot An analytical technique in which antibodies are used to detect the presence of an antigen bound to (i.e., blotted on) a solid matrix such as filter paper (also known as a Western blot).

Immunodeficiency *See* **Acquired immunodeficiency** and **Primary immunodeficiency.**

Immunodominant epitope The epitope of a protein antigen that elicits most of the T cell response in an individual immunized with the native protein. Immunodominant epitopes correspond to the peptides of the protein that are proteolytically generated within APCs, bind most avidly to MHC molecules, and are most likely to stimulate T cells.

Immunofluorescence A technique in which a molecule is detected by use of an antibody labeled with a fluorescent probe. For example, in immunofluorescence microscopy, cells that express a particular surface antigen can be stained with a fluorescein-conjugated antibody specific for the antigen and then visualized with a fluorescent microscope.

Immunogen An antigen that induces an immune response. Not all antigens are immunogens. For example, low-molecular-weight compounds (haptens) can bind to antibodies (and are therefore antigens) but will not stimulate an immune response unless they are linked to macromolecules (carriers).

Immunoglobulin (Ig) Synonymous with antibody (*see* **Antibody**).

Immunoglobulin domain A three-dimensional globular structural motif found in many proteins in the immune system, including Igs, TCRs, and MHC molecules. Ig domains are approximately 110 amino acid residues in length, include an internal disulfide bond, and contain two layers of β-pleated sheets, each layer composed of three to five strands of antiparallel polypeptide chain. Ig domains are classified as V-like or C-like on the basis of closest homology to either the Ig V or C domains.

Immunoglobulin heavy chain One of two types of polypeptide chains in an antibody molecule. The basic structural unit of an antibody includes two identical disulfide-linked heavy chains and two identical light chains. Each heavy chain is composed of a variable (V) Ig domain and three or four constant (C) Ig domains. The different antibody isotypes, including IgM, IgD, IgG, IgA, and IgE, are distinguished by structural differences in their heavy chain constant regions. The heavy chain constant regions also mediate effector functions, such as complement activation or engagement of phagocytes.

Immunoglobulin light chain One of two types of polypeptide chains in an antibody molecule. The basic structural unit of an antibody includes two identical light chains, each disulfide linked to one of two identical heavy chains. Each light chain is composed of one variable (V) Ig domain and one constant (C) Ig domain. There are two light chain isotypes, called κ and λ. Approximately 60% of human antibodies have κ light chains, and 40% have λ light chains.

Immunoglobulin superfamily A large family of proteins that contain a globular structural motif called an Ig domain, or Ig fold, originally described in antibodies. Many proteins of importance in the immune system, including antibodies, TCRs, MHC molecules, CD4, and CD8, are members of this superfamily.

Immunohistochemistry A technique to detect the presence of an antigen in histologic tissue sections by use of an enzyme-coupled antibody that is specific for the antigen. The enzyme converts a colorless substrate to a colored insoluble substance that precipitates at the site where the antibody and thus the antigen are localized. The position of the colored precipitate, and therefore the antigen, in the tissue section is observed by conventional light microscopy. Immunohistochemistry is a routine technique in diagnostic pathology and research.

Immunologic tolerance *See* **Tolerance.**

Immunologically privileged site A site in the body that is inaccessible to or suppresses immune responses. The anterior chamber of the eye, the testes, and the brain are examples of immunologically privileged sites.

Immunoperoxidase technique A common immunohistochemical technique in which a horseradish peroxidase–coupled antibody is used to identify the presence of an antigen in a tissue section. The peroxidase enzyme converts a colorless substrate to an insoluble brown product that is observable by light microscopy.

Immunoprecipitation A technique for the isolation of a molecule from a solution by binding it to an antibody and then rendering the antigen-antibody complex insoluble, either by precipitation with a second antibody or by coupling the first antibody to an insoluble particle or bead.

Immunoreceptor tyrosine-based activation motif (ITAM) A conserved protein motif composed of two copies of the sequence tyrosine-x-x-leucine (where x is an unspecified amino acid) found in the cytoplasmic tails of various membrane proteins in the immune system that are involved in signal transduction. ITAMs are present in the ζ and CD3 proteins of the TCR complex, in Igα and Igβ proteins in the BCR complex, and in several Fc receptors. When these receptors bind their ligands, the tyrosine residues of the ITAMs become phosphorylated and form docking sites for other molecules involved in propagating cell-activating signal transduction pathways.

Immunoreceptor tyrosine-based inhibition motif (ITIM) A six-amino-acid (isoleucine-x-tyrosine-x-x-leucine) motif found in the cytoplasmic tails of various inhibitory receptors in the immune system, including FcγRIIB on B cells and killer cell Ig-like receptors (KIRs) on NK cells. When these receptors bind their ligands, the ITIMs become phosphorylated on their tyrosine residues and form a docking site for tyrosine phosphatases, which in turn function to inhibit other signal transduction pathways.

Immunoreceptor tyrosine-based switch motif (ITSM) A six-amino-acid (tyrosine-x-tyrosine-x-x-valine/isoleucine) motif found in the cytoplasmic tails of some receptors that can sometimes function in an inhibitory way by binding tyrosine phosphatases, as with the ITSM in the cytosolic tail of PD-1 and in other receptors (e.g., in the SLAM family), and can switch from tyrosine phosphatase to tyrosine kinase binding, thereby mediating a change from an inhibitory to an activating function.

Immunosuppression Inhibition of one or more components of the adaptive or innate immune system as a result of an underlying disease or intentionally induced by drugs for the purpose of preventing or treating graft rejection or autoimmune disease. A commonly used immunosuppressive drug is cyclosporine, which blocks T cell cytokine production.

Immunotherapy The treatment of a disease with therapeutic agents that promote or inhibit immune responses. For example, cancer immunotherapy involves promotion of active immune responses to tumor antigens or administration of antitumor antibodies or T cells to establish passive immunity.

Immunotoxins Reagents that may be used in the treatment of cancer and consist of covalent conjugates of a potent cellular toxin derived from plants or bacteria, with antibodies specific for antigens expressed on the surface of tumor cells. Such reagents can specifically target and kill tumor cells without damaging normal cells and are now used to treat several tumors.

Inbred mouse strain A strain of mice created by repetitive mating of siblings that is characterized by homozygosity at every genetic locus. Every mouse of an inbred strain shares an identical set of inherited genes and is said to be syngeneic to every other mouse of the same strain.

Indirect antigen presentation (or indirect allorecognition) In transplantation immunology, a pathway of presentation of donor (allogeneic) MHC molecules by recipient APCs that involves the same mechanisms used to present microbial proteins. The allogeneic MHC proteins are processed by recipient APCs, and peptides derived from the allogeneic MHC molecules are presented, in association with recipient (self) MHC molecules, to host T cells. In contrast to indirect antigen presentation, direct antigen presentation involves recipient T cell recognition of unprocessed allogeneic MHC molecules on the surface of graft cells.

Inflammasome One of a variety of multiprotein complexes that assemble in the cytosol of macrophages, dendritic cells, and other cell types, which proteolytically generate the active form of the inflammatory cytokines IL-1β and IL-18 from inactive precursors. The formation of inflammasomes is stimulated by a variety of microbial products and cell damage–associated molecules and involves assembly of multiple copies of an innate recognition protein with adaptor proteins and procaspase-1 molecules, the latter undergoing proteolysis on inflammasome assembly to generate active caspase-1.

Inflammation A reaction of vascularized tissue to infection or cell injury that involves extravascular accumulation of plasma proteins and leukocytes. Acute inflammation is a common result of innate immune responses, and local adaptive immune responses can also promote inflammation. Although inflammation serves a protective function in controlling infections and promoting tissue repair, it can also cause tissue damage and disease.

Inflammatory bowel disease (IBD) A group of disorders, including ulcerative colitis and Crohn’s disease, characterized by chronic inflammation in the gastrointestinal tract. The cause of IBD is not known, but some evidence indicates that it is caused by inadequate regulation of T cell and innate immune responses, probably against intestinal commensal bacteria.

Innate immunity Protection against infection that relies on mechanisms that exist before infection, are capable of a rapid response to microbes, and react in essentially the same way to repeated infections. The innate immune system includes epithelial barriers, phagocytic cells (neutrophils, macrophages), NK cells, the complement system, and cytokines, largely made by dendritic cells and mononuclear phagocytes, that regulate and coordinate many activities of the cells of innate immunity.

Innate lymphoid cells (ILCs) Tissue-resident cells that produce cytokines similar to those made by helper T cells but lack antigen-specific TCRs. They arise from the common lymphoid progenitor in the bone marrow, have a lymphocyte morphology, and may perform effector functions similar to those of T cells. NK cells are one type of ILC with similar functions to CTLs. Three subsets of helper innate lymphoid cells, called ILC1, ILC2, and ILC3, produce cytokines and express different transcription factors analogous to the Th1, Th2, and Th17 subsets of $CD4^+$ effector T lymphocyte.

Integrins Heterodimeric cell surface proteins whose major functions are to mediate the adhesion of cells to other cells or to extracellular matrix. Integrins are important for T cell interactions with APCs and for migration of leukocytes from blood into tissues. Signals induced by chemokines binding to chemokine receptors increase the affinity of integrins for their ligands. Two examples of integrins important in the immune system are very late antigen 4 (VLA-4), and leukocyte function-associated antigen 1 (LFA-1).

Interferon regulatory factors (IRFs) A family of transcription factors that are important in expression of inflammatory and antiviral genes. For example, IRF3 is activated by TLR signals and stimulates production of type I IFNs, which are cytokines that protect cells from viral infection.

Interferons (IFNs) Cytokines originally named for their ability to interfere with viral infections but that have other important immunomodulatory functions. Type I IFNs include IFN-α and IFN-β, whose main function is to prevent viral replication in cells. IFN-γ, a type II IFN, activates macrophages and various other cell types (*see* Appendix II).

Interleukins (ILs) Any of a large number of cytokines named with a numerical suffix roughly sequentially in order of discovery or molecular characterization (e.g., IL-1, IL-2). Some cytokines were originally named for their biologic activities and do not have an IL designation (*see* Appendix II).

Intracellular bacterium A bacterium that survives and may replicate within cells, usually in endosomes of phagocytes. The principal defense against intracellular bacteria, such as *Mycobacterium tuberculosis,* is T cell–mediated immunity.

Intraepithelial lymphocytes T lymphocytes present in the epidermis of the skin and in mucosal epithelia that typically express a limited diversity of antigen receptors. Some of these lymphocytes, called invariant natural killer T (NKT) cells, may recognize microbial products, such as glycolipids, associated with nonpolymorphic class I MHC-like molecules. Others, called γδ T cells, recognize various nonpeptide antigens, not presented by MHC molecules. Intraepithelial T lymphocytes may be considered effector cells of innate immunity and function in host defense by secreting cytokines and activating phagocytes and by killing infected cells.

Invariant chain (I_i) A nonpolymorphic protein that binds to newly synthesized class II MHC molecules in the endoplasmic reticulum. The invariant chain prevents loading of the class II MHC peptide-binding cleft with peptides present in the endoplasmic reticulum, promotes folding and assembly of class II molecules, and directs them to the endosomal compartment, where loading of peptides derived from internalized proteins takes place.

Isotype One of five types of antibodies, determined by which of five different forms of heavy chain is present. Antibody isotypes (also called classes) include IgM, IgD, IgG, IgA, and IgE, and each isotype performs a different set of effector functions. Additional structural variations characterize four distinct subtypes of IgG and two of IgA.

J

J (joining) chain A small polypeptide that is disulfide linked to the tail pieces of multimeric IgM and IgA antibodies and contributes to the transepithelial transport of these Igs.

JAK-STAT signaling pathway A signaling pathway initiated by cytokine binding to type I and type II cytokine receptors, which sequentially involves activation of receptor-associated Janus kinase (JAK) tyrosine kinases, JAK-mediated tyrosine phosphorylation of the cytoplasmic tails of cytokine receptors, docking of signal transducers and activators of transcription (STATs) to the phosphorylated receptor chains, JAK-mediated tyrosine phosphorylation of the associated STATs, dimerization and nuclear translocation of the STATs, and STAT binding to regulatory regions of target genes causing transcriptional activation of those genes.

Janus kinases (JAKs) A family of four related tyrosine kinases that associate with the cytoplasmic tails of several different cytokine receptors, including the receptors for IL-2, IL-3, IL-4, IL-7, IFN-γ, IL-12, and others. In response to cytokine binding and receptor dimerization, JAKs phosphorylate the cytokine receptors to permit the binding of STATs, and then the JAKs phosphorylate and thereby activate the STATs. Different JAKs associate with different cytokine receptors.

Joining (J) segments Short coding sequences between the variable (V) and constant (C) gene segments in all Ig and TCR loci, which together with D segments are somatically recombined with V segments during lymphocyte development. The resulting recombined VDJ DNA codes for the carboxyl-terminal ends of the antigen receptor V regions, including the third hypervariable (CDR) regions. Random use of different J segments contributes to the diversity of the antigen receptor repertoire.

Junctional diversity The diversity in antibody and TCR repertoires that results from the addition or removal of nucleotide sequences at junctions between V, D, and J gene segments.

K

Kaposi sarcoma A malignant tumor of vascular cells that was frequent in patients with AIDS prior to the use of effective antiretroviral therapy. Kaposi sarcoma is caused by infection with the Kaposi sarcoma–associated herpesvirus (human herpesvirus 8).

Killer cell Ig-like receptors (KIRs) Ig superfamily receptors expressed by NK cells that recognize different alleles of HLA-A, HLA-B, and HLA-C molecules. Some KIRs have signaling components with ITIMs in their cytoplasmic tails, and these deliver inhibitory signals to inactivate the NK cells. Some members of the KIR family have short cytoplasmic tails without ITIMs but associate with other ITAM-containing polypeptides and function as activating receptors.

Knockout mouse A mouse with a targeted disruption of one or more genes that is created by homologous recombination or CRISPR-Cas9 gene editing techniques. Knockout mice lacking functional genes encoding cytokines, cell surface receptors, signaling molecules, and transcription factors have provided extensive information about the roles of these molecules in the immune system.

L

Lamina propria A layer of loose connective tissue underlying epithelium in mucosal tissues such as the intestines and airways, where dendritic cells, mast cells, lymphocytes, and macrophages mediate immune responses to invading pathogens.

Langerhans cells Dendritic cells derived from embryonic macrophages and located as a meshwork in the epidermal layer of the skin whose major function is to capture microbes and antigens that enter through the skin and transport the antigens to draining lymph nodes. During their migration to the lymph nodes, Langerhans cells differentiate into mature dendritic cells, which can efficiently present antigen to naive T cells.

Large granular lymphocyte Another name for an NK cell based on the morphologic appearance of this cell type in the blood.

Late-phase reaction A component of the immediate hypersensitivity reaction that ensues 2 to 4 hours after mast cell degranulation and that is characterized by an inflammatory infiltrate of eosinophils, basophils, neutrophils, and lymphocytes. Repeated bouts of this late-phase inflammatory reaction can cause tissue damage.

LCK A SRC-family nonreceptor tyrosine kinase that non-covalently associates with the cytoplasmic tails of CD4 and CD8 molecules in T cells and is involved in the early signaling events of antigen-induced T cell activation. LCK mediates tyrosine phosphorylation of the cytoplasmic tails of CD3 and ζ proteins of the TCR complex.

Lectin pathway of complement activation A pathway of complement activation triggered by the binding of microbial polysaccharides to the circulating protein mannose binding lectin (MBL). MBL is structurally similar to C1q and activates the C1r-C1s enzyme complex (like C1q) or activates another serine esterase, called mannose-binding protein–associated serine esterase (MASP). The remaining steps of the lectin pathway, beginning with cleavage of C4, are the same as the classical pathway. This pathway can also be initiated by other collectins and ficolins beyond MBL.

Leishmania An obligate intracellular protozoan parasite (usually referring to *Leishmania donovani*) that infects macrophages and can cause a chronic inflammatory disease involving many tissues. *Leishmania* infection in mice has served as a model system for study of the effector functions of several cytokines and the helper T cell subsets that produce them.

Lethal hit A term used to describe the events that result in irreversible damage to a target cell when a CTL binds to it. The lethal hit includes CTL granule exocytosis and perforin-dependent delivery of apoptosis-inducing granule enzymes (granzymes) into the target cell cytoplasm.

Leukemia A malignant disease of bone marrow precursors of blood cells in which large numbers of leukemic cells usually occupy the bone marrow and often circulate in the blood stream. Lymphocytic leukemias are derived from B or T cell precursors, myelogenous leukemias are derived from granulocyte or monocyte precursors, and erythroid leukemias are derived from red blood cell precursors.

Leukocyte adhesion deficiency (LAD) One of a rare group of immunodeficiency diseases with infectious complications that is caused by defective expression or function of the leukocyte adhesion molecules required for tissue recruitment of phagocytes and lymphocytes. LAD-1 is due to mutations in the gene encoding the CD18 protein, which is part of β2 integrins. LAD-2 is caused by mutations in a gene that encodes a fucose transporter involved in the synthesis of ligands for selectins. LAD-3 is due to mutations of genes required for chemokine-induced activation of integrins.

Leukotrienes A class of arachidonic acid–derived lipid inflammatory mediators produced by the lipoxygenase pathway in many cell types. Mast cells make abundant leukotriene C_4 (LTC_4) and its degradation products LTD_4 and LTE_4, which bind to specific receptors on smooth muscle cells and cause prolonged bronchoconstriction. Leukotrienes contribute to the pathologic processes of bronchial asthma.

Lipopolysaccharide *See* **endotoxin.**

Live virus vaccine A vaccine composed of a live but nonpathogenic (attenuated) form of a virus. Attenuated viruses carry mutations that interfere with the viral life cycle or pathogenesis. Because live virus vaccines actually infect the recipient cells, they can effectively stimulate immune responses that are optimal for protecting against wild-type viral infection. A commonly used live virus vaccine is the Sabin oral polio vaccine.

Lymph Interstitial fluid that drains into lymphatic vessels and enters the blood. Lymph carries soluble antigens and dendritic cells from most tissues and organs of the body into lymph nodes for immune surveillance by recruiting lymphocytes and also carries lymphocytes out of lymph nodes and into the circulation.

Lymph node Small, nodular, encapsulated lymphocyte-rich organs situated along lymphatic channels throughout the body where adaptive immune responses to lymph-borne antigens are initiated. Lymph nodes, which are secondary (or peripheral) lymphoid organs, have a specialized anatomic architecture that regulates the interactions of B cells, T cells, dendritic cells, and antigens to maximize the induction of protective immune responses. Lymph nodes contain many macrophages and also perform a filtering function, trapping microorganisms and other potentially harmful constituents in tissue fluids from draining by the lymph into the blood.

Lymphatic system A system of vessels throughout the body that collects tissue fluid called lymph, originally derived in part from the blood, and returns it, through the thoracic duct, to the circulation. Lymph nodes are interspersed along these vessels and trap and retain antigens present in the lymph.

Lymphocyte homing The directed migration of subsets of circulating lymphocytes into particular tissue sites. Lymphocyte homing is regulated by the selective expression of endothelial adhesion molecules and chemokines in different tissues. For example, some lymphocytes preferentially home to the intestinal mucosa, which is regulated by the chemokine CCL25 and the endothelial adhesion molecule MAdCAM, both expressed in the gut, which bind respectively to the CCR9 chemokine receptor and the $\alpha_4\beta_7$ integrin on gut-homing lymphocytes.

Lymphocyte maturation The process by which pluripotent bone marrow stem cells develop into mature, antigen receptor–expressing naive B or T lymphocytes that populate peripheral lymphoid tissues. This process takes place in the specialized environments of the bone marrow (for B cells) and the thymus (for T cells). Synonymous with **lymphocyte development.**

Lymphocyte migration The movement of lymphocytes from the circulation into peripheral tissues.

Lymphocyte recirculation The continuous movement of naive lymphocytes and some memory cells from the blood to secondary lymphoid organs, and back into the blood.

Lymphocyte repertoire The complete collection of antigen receptors and therefore antigen specificities expressed by all the B and T lymphocyte clones of an individual. The repertories for B and T cells are each estimated to be at least 10^7.

Lymphoid follicle A B cell–rich region of a lymph node, spleen, or mucosal lymphoid tissue that is the site of antigen-induced B cell proliferation and differentiation. In T cell–dependent B cell responses to protein antigens, a germinal center forms within the follicles.

Lymphoid tissue inducer (LTi) cells A type of hematopoietically derived innate lymphoid cell that stimulates the development of lymph nodes and other secondary lymphoid organs, in part through production of lymphotoxin, heterotrimers made up of lymphotoxin-α (LTα) and lymphotoxin-β (LTβ). LTis are subtypes of group 3 innate lymphoid cells (ILC3s).

Lymphokine An old name for a cytokine (soluble protein mediator of immune responses) produced by lymphocytes.

Lymphokine-activated killer (LAK) cells NK cells with enhanced cytolytic activity for tumor cells as a result of exposure to high doses of IL-2. LAK cells generated in vitro have been adoptively transferred back into patients with cancer to treat their tumors.

Lymphoma A malignant tumor of B or T lymphocytes usually arising in and spreading between lymphoid tissues but that may spread to other tissues. Lymphomas often express phenotypic characteristics of the normal lymphocytes from which they were derived.

Lysosome A membrane-bound, acidic organelle abundant in phagocytic cells that contains proteolytic enzymes that degrade proteins derived both from the extracellular environment and from within the cell. Lysosomes are involved in the class II MHC pathway of antigen processing.

M

M cells Specialized gastrointestinal mucosal epithelial cells overlying Peyer's patches in the gut that play a role in delivery of antigens to Peyer's patches.

M1 macrophages *See* **Classical macrophage activation.**

M2 macrophages *See* **Alternative macrophage activation.**

Macrophage A hematopoietically derived phagocytic cell that plays important roles in innate and adaptive immune responses. Macrophages are activated by microbial products such as endotoxin and by T cell cytokines such as IFN-γ. Activated macrophages phagocytose and kill microorganisms, secrete proinflammatory cytokines, and present antigens to helper T cells. Macrophages include cells derived from recently recruited blood monocytes at sites of inflammation and long-lived tissue-resident cells derived mainly from fetal hematopoietic organs.

Tissue macrophages are given different names and may serve special functions; these include the microglia of the central nervous system, Kupffer cells in the liver, alveolar macrophages in the lung, and osteoclasts in bone.

Major histocompatibility complex (MHC) A large genetic locus (on human chromosome 6 and mouse chromosome 17) that includes the highly polymorphic genes encoding the peptide-binding molecules recognized by T lymphocytes. The MHC locus also includes genes encoding cytokines, molecules involved in antigen processing, and complement proteins.

Major histocompatibility complex (MHC) molecule A heterodimeric membrane protein encoded in the MHC locus that serves as a peptide display molecule for recognition by T lymphocytes. Two structurally distinct types of MHC molecules exist. Class I MHC molecules are present on most nucleated cells, bind peptides derived from cytosolic proteins that are degraded in proteasomes, and are recognized by $CD8^+$ T cells. Class II MHC molecules are restricted largely to dendritic cells, macrophages, and B lymphocytes; bind peptides derived from endocytosed proteins that are degraded in lysosomes; and are recognized by $CD4^+$ T cells.

Mannose-binding lectin (MBL) A plasma protein that binds to mannose residues on microbial surfaces, thereby initiating the lectin pathway of complement activation. Macrophages express a surface receptor for C1q that can also bind MBL and mediate uptake of the MBL-opsonized organisms.

Mannose receptor A carbohydrate-binding receptor (lectin) expressed by macrophages that binds mannose and fucose residues on microbial cell walls and mediates phagocytosis of the organisms.

Marginal zone A peripheral region of splenic lymphoid follicles containing macrophages that are particularly efficient at trapping polysaccharide antigens. Such antigens may persist for prolonged periods on the surfaces of marginal zone macrophages, where they are recognized by specific B cells, or they may be transported into follicles.

Marginal zone B lymphocytes A subset of B lymphocytes, found in the marginal zone of the spleen, that respond rapidly to blood-borne microbial antigens by producing IgM antibodies with limited diversity.

Mass cytometry A method of simultaneous detection and analysis of many different molecules expressed in mixed cell populations, requiring a specialized instrument based on the single cell analysis of a flow cytometer coupled with a time-of-flight mass spectrometer (CyTOF). This technique uses antibodies labeled with different heavy metal isotopes, rather than fluorochromes used in flow cytometry.

Mast cell The major effector cell of immediate hypersensitivity (allergic) reactions. Mast cells are derived from the bone marrow, reside in most tissues adjacent to blood vessels, express a high-affinity Fc receptor for IgE, and contain numerous mediator-filled granules. Antigen-induced cross-linking of IgE bound to the mast cell Fcε receptors causes release of their granule contents as well as new synthesis and secretion of other mediators, leading to an immediate hypersensitivity reaction.

Mature B cell IgM- and IgD-expressing, functionally competent naive B cells that represent the final stage of B cell maturation in the bone marrow and that populate secondary lymphoid organs.

Medullary thymic epithelial cells (MTECs) A type of stromal cell in the medulla of the thymus that plays a critical role in inducing central T cell tolerance to proteins normally expressed only in certain tissues, called tissue-restricted antigens (TRAs). MTECS express many TRAs under the control of the **AIRE** protein and present these antigens to developing T cells, resulting in death of TRA-specific T cells or development of TRA-specific regulatory T cells.

Membrane attack complex (MAC) A lytic complex of the terminal components of the complement cascade, including complement proteins C5, C6, C7, C8 and multiple copies of C9, which forms in the membranes of target cells. The MAC causes lethal ionic and osmotic changes in cells.

Memory The property of the adaptive immune system to respond more rapidly, with greater magnitude, and more effectively to a repeated exposure to an antigen compared with the response to the first exposure.

Memory lymphocytes Memory B and T cells are produced by antigen stimulation of naive lymphocytes and survive in a functionally quiescent state for many years after the antigen is eliminated. Memory lymphocytes mediate rapid and enhanced (i.e., memory or recall) responses to second and subsequent exposures to antigens.

MHC restriction The characteristic of T lymphocytes that they recognize a foreign peptide antigen only when it is bound to a particular allelic form of an MHC molecule.

β2-Microglobulin The light chain of a class I MHC molecule. β2-Microglobulin is encoded by a nonpolymorphic gene outside the MHC, is structurally homologous to an Ig domain, and is invariant among all class I molecules.

Mitogen-activated protein (MAP) kinase cascade One of several different intracellular signal transduction cascades usually initiated by ligand binding to a membrane receptor, which are characterized by successive kinase-mediated phosphorylation steps leading to the terminal activation by dual phosphorylation of one of a broad family of MAP kinases. The activated MAP kinase then phosphorylates substrates such as transcription factors that result in a functional cellular response. In T lymphocytes, antigen binding to the TCR initiates a MAP kinase cascade that involves the RAS protein and the sequential activation of three kinases, the last one being the MAP kinase called ERK-1,2.

Mixed leukocyte reaction (MLR) An in vitro reaction of alloreactive T cells from one individual against MHC antigens on blood cells from another individual. The MLR involves proliferation of and cytokine secretion by both $CD4^+$ and $CD8^+$ T cells.

Molecular mimicry A postulated mechanism of autoimmunity triggered by infection with a microbe containing antigens that are structurally homologous to and therefore cross-react with self antigens. Immune responses to the microbe are postulated to result in reactions against self antigens.

Monoclonal antibody An antibody specific for one antigen that is produced by a B cell hybridoma (a cell line derived by the fusion of a single normal B cell and an immortal B cell tumor line) or by phage display technology. Monoclonal antibodies are widely used in research, clinical diagnosis, and therapy.

Monocyte A type of bone marrow–derived circulating blood cell that is recruited into sites of infection or tissue injury. Once in the tissue, monocytes differentiate into macrophages that function to fight infections and repair injury.

Mononuclear phagocytes Cells with a common bone marrow lineage whose primary function is phagocytosis. These cells serve various functions in the recognition and activation phases of adaptive immune responses and as effector cells in innate and adaptive immunity. Mononuclear phagocytes circulate in the blood in an incompletely differentiated form called monocytes, and after they settle in tissues, they mature into macrophages.

Mechanistic target of rapamycin (mTOR) Either of two related cytosolic serine/threonine kinases, called mTORC1 and mTORC2. mTORC1 is involved in regulation of many cell functions, including growth, cell cycle, metabolism, and apoptosis. mTOR is activated by AKT and is inhibited by the drug rapamycin.

Mucosa-associated lymphoid tissue (MALT) Collections of lymphocytes, dendritic cells, and other cell types within the mucosa of the gastrointestinal and respiratory tracts that are sites of adaptive immune responses to antigens. Mucosa-associated lymphoid tissues are unencapsulated but organized collections of lymphocytes, with T and B cell zones similar to lymph nodes, below mucosal epithelia, such as Peyer's patches in the gut or pharyngeal tonsils.

Mucosa-associated invariant T (MAIT) cells A subset of T cells that express an invariant $\alpha\beta$ TCR specific for fungal and bacterial riboflavin metabolites presented by a nonpolymorphic class I MHC–related molecule called MR1. Most MAIT cells are $CD8^+$, are activated either by microbial riboflavin derivatives or by cytokines, and have inflammatory and cytotoxic functions. MAIT cells account for about 50% of all T cells in the human liver.

Mucosal immune system A part of the immune system that responds to and protects against microbes that enter the body through mucosal surfaces, such as the gastrointestinal and respiratory tracts, but also maintains tolerance to commensal organisms that live on the outside of the mucosal epithelium. The mucosal immune system is composed of organized mucosa-associated lymphoid tissues, such as Peyer's patches, as well as diffusely distributed cells within the lamina propria.

Multiple myeloma A malignant tumor of plasma cells that often secretes intact antibodies or parts of antibody molecules. The monoclonal antibodies produced by multiple myelomas were critical for early biochemical analyses of antibody structure.

Multivalency *See* **Polyvalency.**

Multiple sclerosis A chronic relapsing or progressive autoimmune disease of the central nervous system characterized by inflammatory damage to the myelin sheath of neurons, mediated by autoreactive $CD4^+$ T cells and macrophages, leading to impairment of sensory and motor functions.

Mycobacterium A genus of aerobic bacteria, many species of which can survive within phagocytes and cause disease. The principal host defense against mycobacteria such as *Mycobacterium tuberculosis* is cell-mediated immunity.

Myeloid-derived suppressor cells (MDSCs) A heterogeneous group of myeloid cells derived from the same precursors that give rise to neutrophils and monocytes, but have antiinflammatory and immunosuppressive properties. MDSCs are found in lymphoid tissues, blood, or tumors of cancer-bearing animals and cancer patients and are thought to suppress antitumor immune responses.

N

N nucleotides The name given to nucleotides randomly added to the junctions between V, D, and J gene segments in *Ig* or *TCR* genes during lymphocyte development. The addition of up to 20 of these nucleotides, which is mediated by the enzyme terminal deoxyribonucleotidyl transferase (TdT), contributes to the diversity of the antibody and TCR repertoires.

Naive lymphocyte A mature B or T lymphocyte that has not previously encountered antigen. When naive lymphocytes are stimulated by antigen, they differentiate into effector lymphocytes, such as antibody-secreting B cells, cytokine-producing helper T cells, and CTLs capable of killing target cells. Naive lymphocytes have surface markers and recirculation patterns that are distinct from those of previously activated lymphocytes. (“Naive” also refers to an unimmunized individual.)

Natural antibodies IgM antibodies produced without overt antigen exposure, largely by B-1 cells, specific for bacteria that are common in the environment and gastrointestinal tract. Normal individuals have natural antibodies without any evidence of infection, and these antibodies may serve as a preformed defense mechanism against microbes that succeed in penetrating epithelial barriers. Antibodies against ABO blood group antigens, which are responsible for transfusion reactions, are natural antibodies.

Natural killer (NK) cells A subset of innate lymphoid cells that function in innate immune responses to kill microbe-infected cells by direct lytic mechanisms and by secreting IFN-γ. NK cells do not express clonally distributed antigen receptors like Ig receptors or TCRs, and their activation is regulated by a combination of cell surface stimulatory and inhibitory receptors, the latter recognizing self MHC molecules.

Natural killer T cells (NKT cells) A numerically small subset of lymphocytes that express TCRs and some surface molecules characteristic of NK cells. Some NKT cells, called invariant NKT (iNKT) cells, express $\alpha\beta$ T cell antigen receptors with very little diversity and recognize lipid antigens presented by CD1 molecules. The physiologic functions of NKT cells are not well defined.

Negative selection The process by which developing lymphocytes that express self-reactive antigen receptors are eliminated, thereby contributing to the maintenance of self-tolerance. Negative selection of developing T lymphocytes (thymocytes) is best understood and involves high-avidity binding of a thymocyte to self MHC molecules with bound peptides on thymic APCs, leading to apoptotic death of the self-reactive thymocyte.

Neoantigen A molecule that is newly changed, either by chemical modification or, in the case of proteins, by mutation of the encoding gene, such that the new structure is recognized by antibodies or T cells. Neoantigens (sometimes called neoepitopes) are the major inducers of tumor-specific T cell responses.

Neonatal Fc receptor (FcRn) An IgG-specific Fc receptor that mediates the transport of maternal IgG across the placenta and the neonatal intestinal epithelium and, in adults, promotes the long half-life of IgG molecules in the blood by protecting them from catabolism in phagocytes and endothelial cells.

Neonatal immunity Passive humoral immunity to infections in mammals in the first months of life, before full development of the immune system. Neonatal immunity is mediated by maternally produced IgG antibodies transported across the placenta into the fetal circulation before birth or derived from ingested milk and transported across the gut epithelium.

Neutrophil (also called polymorphonuclear leukocyte [PMN]) A phagocytic cell characterized by a segmented lobular nucleus and cytoplasmic granules filled with degradative enzymes. Neutrophils are the most abundant type of circulating white blood cells and are the most numerous cell type recruited into tissues as part of acute inflammatory responses to microbial infections.

Nitric oxide A molecule with a broad range of activities that in macrophages functions as a potent microbicidal agent to kill ingested organisms.

Nitric oxide synthase A member of a family of enzymes that synthesize the vasoactive and microbicidal compound nitric oxide from L-arginine. Macrophages express an inducible form of this enzyme on activation by various microbial or cytokine stimuli.

NOD-like receptors (NLRs) A family of cytosolic multidomain proteins that sense cytoplasmic PAMPs and DAMPs and recruit other proteins to form signaling complexes that promote inflammation.

Notch-1 A cell surface signaling receptor that is proteolytically cleaved after ligand binding and whose cleaved intracellular portion translocates to the nucleus and regulates gene expression. Notch-1 signaling is required for commitment of developing T cell precursors to the αβ T cell lineage.

Nuclear factor κB (NF-κB) A family of transcription factors composed of homodimers or heterodimers of proteins homologous to the c-REL protein. NF-κB proteins are required for the inducible transcription of many genes important in both innate and adaptive immune responses.

Nuclear factor of activated T cells (NFAT) A transcription factor required for the expression of IL-2, IL-4, TNF, and other cytokine genes. Four different NFATs are each encoded by separate genes;

NFATp and NFATc are found in T cells. Cytoplasmic NFAT is activated by calcium/calmodulin-dependent, calcineurin-mediated dephosphorylation that permits NFAT to translocate into the nucleus and bind to consensus binding sequences in the regulatory regions of IL-2, IL-4, and other cytokine genes, usually in association with other transcription factors such as AP-1.

Nude mouse A strain of hairless mice that lacks development of the thymus, and therefore T lymphocytes, as well as hair follicles, caused by mutation affecting the transcription factor FOXN1. Nude mice have been used experimentally to define the role of T lymphocytes in immunity and disease.

O

Oncofetal antigen Proteins that are expressed at high levels on some types of cancer cells and in normal developing fetal (but not adult) tissues. Antibodies specific for these proteins are used in histopathologic identification of tumors, and serum levels of these proteins are assayed to monitor the progression of tumor growth in patients. Carcinoembryonic acid (CEA) (CD66) and α-fetoprotein are two oncofetal antigens commonly expressed by certain carcinomas.

Opsonin A molecule that becomes attached to the surface of a microbe and can be recognized by surface receptors of neutrophils and macrophages, thereby increasing the efficiency of phagocytosis of the microbe. Opsonins include IgG antibodies, which are recognized by the Fcγ receptor on phagocytes, and fragments of the C3 complement protein, which are recognized by CR1 (CD35) and by the leukocyte integrin MAC-1.

Opsonization The process of attaching opsonins, such as IgG or complement fragments, to microbial surfaces to target the microbes for phagocytosis.

Oral tolerance The suppression of systemic humoral and cell-mediated immune responses to an antigen after the oral administration of that antigen. It may be the result of anergy of antigen-specific T cells or the production of immunosuppressive cytokines such as transforming growth factor-β. Oral tolerance is a possible mechanism for prevention of immune responses to food antigens and to bacteria that normally reside as commensals in the intestinal lumen.

P

P nucleotides Short inverted repeat nucleotide sequences in the VDJ junctions of rearranged *Ig* and *TCR* genes that are added after ARTEMIS-mediated RAG-1- and RAG-2-mediated asymmetric cleavage of hairpin DNA intermediates during somatic recombination events. P nucleotides contribute to the junctional diversity of antigen receptors.

Paracrine factor A molecule that acts on cells in proximity to the cell that produces the factor. Most cytokines act in a paracrine fashion.

Passive immunity The form of immunity to an antigen that is established in one individual by transfer of antibodies or lymphocytes from another individual who is immune to that antigen. The recipient of such a transfer can become immune to the antigen without ever having been exposed to or having responded to the antigen. Transplacental transfer of IgG from mother to fetus is a physiologic form of passive immunity essential for health of newborn babies. Examples of therapeutic passive immunity are the administration of human sera or plasma containing antibodies specific for potentially lethal microbial toxins, snake venom, or microbes in individuals exposed to those toxins or microbes. Treatments with synthetic monoclonal antimicrobial antibodies have also been developed.

Pathogen-associated molecular patterns (PAMPs) Structures produced by microorganisms, but not mammalian (host) cells, that are recognized by and stimulate the innate immune system. Examples include bacterial lipopolysaccharide and viral double-stranded RNA.

Pathogenicity The ability of a microorganism to cause disease. Multiple mechanisms may contribute to pathogenicity, including production of toxins, stimulation of host inflammatory responses, and perturbation of host cell metabolism.

Pattern recognition receptors Signaling receptors of the innate immune system that recognize PAMPs and DAMPs, and thereby activate innate immune responses. Examples include TLRs and NOD-like receptors (NLRs).

PD-1 An inhibitory receptor homologous to CD28 that is expressed on activated T cells and binds to its ligands PD-L1 or PD-L2, members of the B7 protein family expressed on various cell types. PD-1 is upregulated on T cells after repeated or prolonged stimulation, such as in the setting of chronic infection or tumors, and blockade of PD-1 with monoclonal antibodies enhances antitumor immune responses.

Pentraxins A family of plasma proteins that contain five identical globular subunits; includes the acute-phase reactant C-reactive protein (CRP).

Peptide-binding cleft The portion of an MHC molecule that binds peptides for display to T cells. The cleft is composed of paired α helices resting on a floor made up of an eight-stranded β-pleated sheet. The polymorphic residues, which are the amino acids that vary among different MHC alleles, are located in and around this cleft.

Peptide-MHC tetramer A reagent used to identify and enumerate T cells that specifically recognize a particular MHC-peptide complex. The reagent consists of four recombinant, biotinylated MHC molecules (usually class I) bound to a fluorochrome-labeled avidin molecule and loaded with a peptide. T cells that bind the MHC tetramer can be detected by flow cytometry.

Perforin A protein present in the granules of CTLs and NK cells. When perforin is released from the granules of activated CTLs or NK cells, it inserts into membrane of the adjacent infected cells and promotes entry of granzymes into the cytosol, leading to apoptotic death of the cell.

Periarteriolar lymphoid sheath (PALS) A cuff of lymphocytes surrounding small arterioles in the spleen, adjacent to lymphoid follicles. The PALS contains mainly T lymphocytes, approximately two-thirds of which are $CD4^+$ and one-third $CD8^+$. In humoral immune responses to protein antigens, B lymphocytes are activated at the interface between the PALS and follicles and then migrate into the follicles to form germinal centers.

Peripheral lymphoid organs and tissues. *See* **Secondary lymphoid organs and tissues.**

Peripheral tolerance Unresponsiveness to self antigens that are present in peripheral tissues and not usually in the generative lymphoid organs. Peripheral tolerance is induced by various mechanisms, including the recognition of antigens without adequate levels of the costimulators required for lymphocyte activation, by persistent and repeated stimulation by self antigens, or by regulatory T cell-mediated suppression.

Peyer's patches Organized lymphoid tissue in the lamina propria of the small intestine in which immune responses to intestinal pathogens and other ingested antigens may be initiated. Peyer's patches are composed mostly of B cells, with smaller numbers of T lymphocytes and other cells, all arranged in follicles similar to those found in lymph nodes, often with germinal centers.

Phagocytosis The process by which certain cells of the innate immune system, including macrophages and neutrophils, engulf large particles (>0.5 μm in diameter), such as intact microbes. The cell surrounds the particle with extensions of its plasma membrane by an energy- and cytoskeleton-dependent process, resulting in the formation of an intracellular vesicle called a phagosome containing the ingested particle. Phagosomes fuse with acidic vesicles of the endolysosomal system that contains enzymes that generate free-radicals and proteases, which mediate killing of ingested microbes.

Phagosome A membrane-bound intracellular vesicle that contains microbes or particulate material ingested from the extracellular environment. Phagosomes are formed during the process of phagocytosis. They fuse with endolysosomal vesicles, leading to degradation of the ingested material.

Phosphatase (protein phosphatase) An enzyme that removes phosphate groups from the side chains of certain amino acid residues of proteins. Protein phosphatases in lymphocytes, such as CD45, calcineurin, SHP1 (SH2 domain-containing protein phosphatase 1), and SHP2, regulate the activity of various signal transduction molecules and transcription factors. Some protein phosphatases may be specific for phosphotyrosine residues and others for phosphoserine and phosphothreonine residues.

Phospholipase Cγ (PLCγ) An enzyme that catalyzes hydrolysis of the plasma membrane phospholipid phosphatidylinositol 4,5-

bisphosphate (PIP2) to generate two signaling molecules, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). PLCγ becomes activated in lymphocytes after antigen binding to the antigen receptor.

Phytohemagglutinin (PHA) A carbohydrate-binding protein, or lectin, produced by plants that cross-links human T cell surface molecules, including the TCR, thereby inducing polyclonal activation and agglutination of T cells. PHA was used in experimental immunology to study T cell activation. In clinical medicine, PHA is used to assess whether a patient's T cells are functional or to induce T cell mitosis for the purpose of generating karyotypic data.

Plasmablast Circulating antibody-secreting cells that are precursors of the plasma cells that reside in the bone marrow and other tissues.

Plasma cell A terminally differentiated antibody-secreting B lymphocyte with a characteristic histologic appearance, including an oval shape, eccentric nucleus with peripheral chromatin distribution, and perinuclear halo. Plasma cells are found in bone marrow, mucosal tissues, and many sites of chronic inflammation.

Platelet-activating factor (PAF) A lipid mediator derived from membrane phospholipids in several cell types, including mast cells and endothelial cells. PAF can cause bronchoconstriction and vascular dilation and leak.

Polyclonal activators Agents that are capable of activating many clones of lymphocytes, regardless of their antigen specificities. Examples of polyclonal activators include anti-IgM antibodies for B cells and anti-CD3 antibodies, bacterial superantigens, and PHA for T cells.

Poly-Ig receptor An Fc receptor expressed by mucosal epithelial cells that mediates the transport of IgA and IgM secreted by plasma cells in the intestinal lamina propria through intestinal epithelial cells into the lumen.

Polymerase chain reaction (PCR) A rapid method of copying and amplifying specific DNA sequences up to about 1 kb in length that is widely used as a preparative and analytical technique in all branches of molecular biology. The method relies on the use of short oligonucleotide primers complementary to the sequences at the ends of the DNA to be amplified and involves repetitive cycles of melting, annealing, and synthesis of DNA.

Polymorphism The existence of two or more alternative forms, or variants, of a gene that are present at stable frequencies in a population. Each common variant of a polymorphic gene is called an allele, and one individual may carry two different alleles of a gene, each inherited from a different parent. The MHC genes, some of which have thousands of alleles, are the most polymorphic genes in the mammalian genome.

Polyvalency The presence of multiple identical epitopes on a single antigen molecule, cell surface, or particle. Polyvalent antigens, such as bacterial capsular polysaccharides, are often capable of activating B lymphocytes independent of helper T cells. Used synonymously with **multivalency.**

Positive selection The process by which developing T cells in the thymus (thymocytes) whose TCRs bind to self MHC molecules are rescued from programmed cell death, whereas thymocytes whose receptors do not recognize self MHC molecules die by default. Positive selection ensures that mature T cells are self MHC restricted and that CD8$^+$ T cells are specific for complexes of peptides with class I MHC molecules and CD4$^+$ T cells for complexes of peptides with class II MHC molecules.

Pre-B cell A developing B cell present only in hematopoietic tissues that is at a maturational stage characterized by expression of cytoplasmic Ig μ heavy chains and surrogate light chains but not Ig light chains. Pre-BCRs composed of μ chains and surrogate light chains deliver signals that stimulate further maturation of the pre-B cell into an immature B cell.

Pre-B cell receptor A receptor expressed on developing B lymphocytes at the pre-B cell stage that is composed of Ig μ heavy chains and invariant surrogate light chains. The pre-BCR associates with the Igα and Igβ signal transduction proteins to form the pre-BCR complex. Pre-BCRs are required for stimulating the proliferation and continued maturation of the developing B cell, serving as a checkpoint that ensures productive μ heavy chain VDJ rearrangement. It is not known whether the pre-BCR binds a specific ligand.

Pre-T cell A developing T lymphocyte in the thymus at a maturational stage characterized by expression of the TCR β chain but not the α chain or CD4 or CD8. In pre-T cells, the TCR β chain is found on the cell surface as part of the pre-TCR.

Pre-T cell receptor A receptor expressed on the surface of pre-T cells that is composed of the TCR β chain and an invariant pre-Tα protein. This receptor associates with CD3 and ζ molecules to form the pre-TCR complex. The function of this complex is similar to that of the pre-BCR in B cell development, namely, the delivery of signals that stimulate further proliferation, antigen receptor gene rearrangements, and other maturational events. The pre-TCR serves as a checkpoint that ensures productive β chain VDJ rearrangement. It is not known whether the pre-TCR binds a specific ligand.

Pre-Tα An invariant transmembrane protein with a single extracellular Ig-like domain that associates with the TCR β chain in pre-T cells to form the pre-TCR.

Primary immune response An adaptive immune response that occurs after the first exposure of an individual to a foreign antigen. Primary responses are characterized by relatively slow kinetics and small magnitude compared with secondary (memory) responses after a second or subsequent exposure.

Primary immunodeficiency disease (PID) A genetic defect in which an inherited deficiency in some aspect of the innate or adaptive immune system leads to an increased susceptibility to infections. Congenital immunodeficiency is frequently manifested early in infancy and childhood but is sometimes clinically detected later in life. Synonymous with **congenital immunodeficiency disease.**

Pro-B cell A developing B cell in the bone marrow that is the earliest cell committed to the B lymphocyte lineage. Pro-B cells do not produce Ig, but they can be distinguished from other immature cells by the expression of B lineage–restricted surface molecules such as CD19 and CD10.

Pro-T cell A developing T cell in the thymic cortex that is a recent arrival from the bone marrow and does not express TCRs, CD3, ζ chains, or CD4 or CD8 molecules. Pro-T cells are also called double-negative thymocytes.

Professional antigen-presenting cells (professional APCs) A term sometimes used to refer to APCs that activate T lymphocytes; includes mainly dendritic cells (DCs), but also mononuclear phagocytes and B lymphocytes, all of which are capable of expressing class II MHC molecules and costimulators. The most important professional APCs for initiating primary T cell responses are DCs.

Programmed cell death *See* **Apoptosis.**

Promoter A DNA sequence immediately 5′ to the transcription start site of a gene where the proteins that initiate transcription bind. The term *promoter* is often used to mean the entire 5′ regulatory region of a gene, excluding enhancers, that are additional sequences that bind transcription factors and interact with the basal transcription complex to increase the rate of transcriptional initiation. Other enhancers may be located at a significant distance from the promoter, either 5′ of the gene, in introns, or 3′ of the gene.

Prostaglandins A class of lipid inflammatory mediators that are derived from arachidonic acid in many cell types through the cyclooxygenase pathway and that have vasodilator, bronchoconstrictor, and chemotactic activities. Prostaglandins made by mast cells are important mediators of allergic reactions. Many commonly used antiinflammatory drugs are cyclooxygenase inhibitors, which block the synthesis of prostaglandins.

Proteasome A large multiprotein enzyme complex with a broad range of proteolytic activity that is found in the cytosol of most cells, important for degrading misfolded cytosolic and nuclear proteins. Proteins are targeted for proteasomal degradation by covalent linkage of ubiquitin molecules. A specialized form of the proteasome formed in APCs, called the immunoproteasome, degrades cytosolic proteins into peptides that are transported into the endoplasmic reticulum and bind to newly synthesized class I MHC molecules.

Protein kinase C (PKC) Any of several isoforms of an enzyme that mediates the phosphorylation of serine and threonine residues in many different protein substrates and thereby serves to propagate various signal transduction pathways leading to transcription factor activation. In T and B lymphocytes, PKC is activated by DAG, which is generated in response to antigen receptor ligation.

Protein tyrosine kinases (PTKs) Enzymes that mediate the phosphorylation of tyrosine residues in proteins and thereby promote phosphotyrosine-dependent protein-protein interactions. PTKs are involved in numerous signal transduction pathways in cells of the immune system.

Protozoa Single-celled eukaryotic organisms, many of which are human parasites and cause diseases. Examples of pathogenic protozoa include *Entamoeba histolytica,* which causes amebic dysentery; *Plasmodium,* which causes malaria; and *Leishmania donovani* which causes leishmaniasis. Protozoa stimulate both innate and adaptive immune responses. It has proved difficult to develop effective vaccines against many of these organisms.

Provirus A DNA copy of the genome of a retrovirus that is integrated into the host cell genome and from which viral genes are transcribed and the viral genome is reproduced. HIV proviruses can remain inactive for long periods and thereby represent a latent form of HIV infection that is not accessible to immune defense.

Purified antigen (subunit) vaccine A vaccine composed of purified antigens or portions (subunits) of antigens. Examples of this type of vaccine include diphtheria and tetanus toxoids, pneumococcus and *Haemophilus influenzae* polysaccharide vaccines, and purified polypeptide vaccines against hepatitis B and influenza virus. Purified antigen vaccines may stimulate antibody and helper T cell responses, but they typically do not generate CTL responses.

Pyogenic bacteria Bacteria, such as gram-positive staphylococci and streptococci, that induce inflammatory responses rich in polymorphonuclear leukocytes (giving rise to pus). Antibody responses to these bacteria greatly enhance the efficacy of innate immune effector mechanisms to clear infections.

Pyroptosis A form of programmed cell death of macrophages and dendritic cells induced by canonical inflammasome activation of caspase-1 (and also noncanonical inflammasome pathways that use human caspase-4 or caspase-5), characterized by cell swelling, loss of plasma membrane integrity, and release of inflammatory mediators, such as IL-1β. In pyroptosis, the activated caspases proteolytically generate a fragment of the protein gasdermin D, which polymerizes to form pores in the plasma membrane. Pyroptosis results in the death of certain microbes that gain access to the cytosol, enhances inflammatory clearance of bacteria, but also contributes to septic shock.

R

Radioimmunoassay A highly sensitive and specific immunologic method of quantifying the concentration of an antigen in a solution that relies on a radioactively labeled antibody specific for the antigen. Usually, two antibodies specific for the antigen are used. The first antibody is unlabeled but attached to a solid support, where it binds and immobilizes the antigen whose concentration is being determined. The amount of the second, labeled antibody that binds to the immobilized antigen, as determined by radioactive decay detectors, is proportional to the concentration of antigen in the test solution. Radioimmunoassays have largely been replaced by non-radioactive solid-phase immunoassays, such as enzyme-linked immunosorbent assays (ELISAs; *see* Appendix III).

Rapamycin An immunosuppressive drug (also called sirolimus) used clinically to prevent allograft rejection. Rapamycin inhibits the activation of a protein called mechanistic target of rapamycin (mTOR), which is a key signaling molecule in a variety of metabolic and cell growth pathways, including the pathway required for IL-2–mediated T cell proliferation.

RAS A member of a family of 21-kD guanine nucleotide-binding proteins with intrinsic GTPase activity that are involved in many different signal transduction pathways in diverse cell types. Mutated *RAS* genes are associated with neoplastic transformation. In T cell activation, RAS is activated by GDP-GTP exchange factors that are recruited to tyrosine phosphorylated adaptors. GTP·RAS then initiates the MAP kinase cascade, which leads to expression of the *FOS* gene and assembly of the AP-1 transcription factor.

Reactive oxygen species (ROS) Highly reactive metabolites of oxygen, including superoxide anion, hydroxyl radical, and hydrogen peroxide, that are produced by activated phagocytes. Reactive oxygen species are used by the phagocytes to form oxyhalides that damage ingested bacteria. They also may be released from cells and promote inflammatory responses or cause tissue damage.

Reagin IgE antibody that mediates an immediate hypersensitivity reaction.

Receptor editing A process by which some immature B cells that recognize self antigens in the bone marrow may be induced to change their Ig specificities. Receptor editing involves reactivation of the *RAG* genes, additional light chain VJ recombinations, and new Ig light chain production, which allows the cell to express a different Ig receptor that is not self-reactive.

Recombination-activating genes 1 and 2 (*RAG1* and *RAG2*) The genes encoding RAG-1 and RAG-2 proteins, which make up the V(D)J recombinase and are expressed in developing B and T cells. RAG proteins bind to recombination signal sequences and are critical for DNA recombination events that form functional *Ig* and *TCR* genes. Therefore, RAG proteins are required for expression of antigen receptors and for the maturation of B and T lymphocytes.

Recombination signal sequences Specific DNA sequences found adjacent to the V, D, and J segments in antigen receptor loci that are recognized by the RAG-1/RAG-2 complex during V(D)J recombination. The recognition sequences consist of a highly conserved stretch of 7 nucleotides, called the heptamer, located adjacent to the V, D, or J coding sequence, followed by a spacer of exactly 12 or 23 nonconserved nucleotides and a highly conserved stretch of 9 nucleotides, called the nonamer.

Red pulp An anatomic and functional compartment of the spleen composed of vascular sinusoids, scattered among which are large numbers of erythrocytes, macrophages, dendritic cells, sparse lymphocytes, and plasma cells. Red pulp macrophages clear the blood of microbes, other foreign particles, and damaged red blood cells.

Regulatory T cells A population of T cells that inhibits the activation of other T cells and is necessary to maintain peripheral tolerance to self antigens. Most regulatory T cells are $CD4^+$ and express the α chain of the IL-2 receptor (CD25), CTLA-4, and the transcription factor FOXP3.

Rejection An adaptive immune response against a tissue or organ graft, usually an allograft. Graft rejection may be mediated by antibodies and/or T cells, and there are different pathologically defined types of rejection, including hyperacute, acute, and chronic.

Respiratory burst The process by which reactive oxygen species such as superoxide anion, hydroxyl radical, and hydrogen peroxide are produced in neutrophils and macrophages. The respiratory burst is mediated by the enzyme phagocyte oxidase and is usually triggered by inflammatory mediators, such as the cytokines IFN-γ and TNF, or by bacterial products, such as lipopolysaccharide.

Reverse transcriptase An enzyme encoded by retroviruses, such as HIV, that synthesizes a DNA copy of the viral genome from the RNA genomic template. Purified reverse transcriptase is used widely in molecular biology research for purposes of cloning complementary DNAs encoding a gene of interest from messenger RNA. Reverse transcriptase inhibitors are used as drugs to treat HIV-1 infection.

Rh blood group antigens A system of protein alloantigens expressed on red blood cell membranes that are the cause of transfusion reactions and hemolytic disease of the fetus and newborn. The most clinically important Rh antigen is designated RhD.

Rheumatoid arthritis An autoimmune disease characterized primarily by inflammatory damage to joints and sometimes inflammation of blood vessels, lungs, and other tissues. $CD4^+$ T cells, activated B lymphocytes, and plasma cells are found in the inflamed joint lining (synovium), and numerous proinflammatory cytokines, including IL-1, IL-6, and TNF, are present in the synovial (joint) fluid.

RIG-like receptors (RLRs) Cytosolic receptors of the innate immune system that recognize viral RNA and induce production of type I IFNs. The two best characterized RLRs are RIG-I (retinoic acid–inducible gene I) and MDA5 (melanoma differentiation–associated gene 5).

RNA vaccine A vaccine comprised of messenger RNA encoding one or more microbial antigens, usually packaged within lipid nanoparticles. RNA vaccines specific for SARS-CoV-2 spike proteins were the first vaccines to be widely used during the COVID-19 pandemic.

RORγT (retinoid-related orphan receptor γ T) A transcription factor expressed in and required for differentiation of Th17 cells and type 3 innate lymphoid cells, encoded by the *RORC* gene.

S

SARS-CoV-2 *See* **COVID-19.**

Scavenger receptors A family of cell surface receptors expressed on macrophages, originally defined as receptors that mediate endocytosis of oxidized or acetylated low-density lipoprotein particles but that also bind and mediate the phagocytosis of a variety of microbes.

SCID mouse A mouse strain in which B and T cells are absent because of an early block in maturation from bone marrow precursors. SCID mice carry a mutation in a component of the enzyme DNA-dependent protein kinase, which is required for double-stranded DNA break repair. Deficiency of this enzyme results in abnormal joining of Ig and TCR gene segments during recombination and therefore failure to express antigen receptors.

Secondary immune response An adaptive immune response that occurs on second exposure to an antigen. A secondary response is characterized by more rapid kinetics and greater magnitude relative to the primary immune response, which occurs on first exposure.

Secondary immunodeficiency *See* **Acquired immunodeficiency.**

Secondary lymphoid organs and tissues Organized collections of lymphocytes and accessory cells, including the spleen, lymph nodes, and mucosa-associated lymphoid tissues, in which adaptive immune responses are initiated; also called **peripheral lymphoid organs and tissues.**

Second-set rejection Allograft rejection in an individual who has previously been sensitized to the donor's tissue alloantigens by having received another graft or transfusion from that donor. In contrast to first-set rejection, which occurs in an individual who has not previously been sensitized to the donor alloantigens, second-set rejection is rapid and occurs in 3 to 7 days as a result of immunologic memory.

Secretory component The proteolytically cleaved portion of the extracellular domain of the poly-Ig receptor that remains bound to an IgA molecule in mucosal secretions.

Selectin Any one of three separate but closely related carbohydrate-binding proteins that mediate low-affinity adhesion of leukocytes to postcapillary venule endothelial cells, leading to rolling of the leukocytes on endothelial surfaces of the venules. Each of the selectin molecules is a single-chain transmembrane glycoprotein with a similar modular structure, including an extracellular calcium-dependent lectin domain. The selectins include L-selectin (CD62L), expressed on leukocytes; P-selectin (CD62P), expressed on platelets and activated endothelium; and E-selectin (CD62E), expressed on activated endothelium.

Selective immunoglobulin deficiency Immunodeficiencies characterized by a lack of only one or a few Ig classes or subclasses. IgA deficiency is the most common selective Ig deficiency, followed by IgG3 and IgG2 deficiencies. Patients with these disorders may be at increased risk for bacterial infections, but many are normal.

Self MHC restriction The limitation (or restriction) of T cells to recognize antigens displayed by MHC molecules that the T cell encountered during maturation in the thymus (and thus sees as self MHC).

Self-tolerance Unresponsiveness of the adaptive immune system to self antigens, largely as a result of inactivation or death of self-reactive lymphocytes induced by exposure to these antigens or suppression by regulatory T cells. Self-tolerance is a cardinal feature of the normal immune system, and failure of self-tolerance leads to autoimmune diseases.

Septic shock A severe complication of bacterial or fungal infections that spread to the blood stream (sepsis) and is characterized by hypotension and shock, disseminated intravascular coagulation, and metabolic disturbances. In bacterial infections, the syndrome is due to the effects of bacterial cell wall components, such as lipopolysaccharide or peptidoglycan, that bind to TLRs on various cell types and induce expression of large amounts of inflammatory cytokines, including TNF, IL-6, and IL-12.

Seroconversion The production of detectable antibodies in the serum specific for a microorganism during the course of an infection or in response to immunization.

Serology The study of blood (serum) antibodies and their reactions with antigens. The term serology is often used to refer to the diagnosis of infectious diseases by detection of microbe-specific antibodies in the serum.

Serotype An antigenically distinct subset of a species of an infectious organism that is distinguished from other subsets by serologic (i.e., serum antibody binding) tests. Humoral immune responses to one serotype of microbes (e.g., influenza virus) may not be protective against another serotype.

Serum The cell-free fluid that remains when blood or plasma forms a clot. Blood antibodies are found in the serum fraction.

Serum amyloid A (SAA) An acute-phase protein whose serum concentration rises significantly in the setting of infection and inflammation, mainly because of IL-1– and TNF-induced synthesis by the liver. SAA activates leukocyte chemotaxis, phagocytosis, and adhesion to endothelial cells.

Serum sickness A disease caused by the injection of large doses of a protein antigen into the blood and characterized by the deposition of antigen-antibody (immune) complexes in blood vessel walls, especially in the kidneys and joints. Immune complex deposition leads to complement fixation and leukocyte recruitment and subsequently to glomerulonephritis and arthritis. Serum sickness was originally described as a disorder that occurred in patients receiving injections of horse serum containing antitoxin antibodies to prevent diphtheria.

Severe combined immunodeficiency (SCID) Immunodeficiency diseases in which both B and T lymphocytes do not develop or do not function properly, and therefore both humoral immunity and cell-mediated immunity are impaired. Children with SCID usually have infections during the first year of life and succumb to these infections unless the immunodeficiency is treated. SCID has several different genetic causes.

Signal transducer and activator of transcription (STAT) A member of a family of seven mammalian proteins that function as transcription factors in response to binding of cytokines to type I and type II cytokine receptors. STATs are present as inactive monomers in the cytosol of cells and are recruited to the cytoplasmic tails of cross-linked cytokine receptors, where they are tyrosine phosphorylated by JAKs. The phosphorylated STAT proteins dimerize and move to the nucleus, where they bind to specific sequences in the promoter regions of various genes and stimulate their transcription. Different STATs are activated by different cytokines.

Single-chain variable fragment (single chain Fv) A genetically engineered single polypeptide that includes a both Ig heavy chain and light chain V domains that fold to form an antibody binding site of known specificity, used as a research reagent or as part of an anti-tumor drug, such as bispecific T cell activators (BiTEs) and chimeric antigen receptors (CARs).

Single-positive thymocyte A maturing T cell precursor in the thymus that expresses CD4 or CD8 molecules but not both. Single-positive thymocytes are found mainly in the medulla and have matured from the double-positive stage, during which thymocytes express both CD4 and CD8 molecules.

Smallpox A disease caused by variola virus. Smallpox was the first infectious disease shown to be preventable by vaccination and the first disease to be completely eradicated by a worldwide vaccination program.

Somatic hypermutation High-frequency point mutations in Ig heavy and light chains that occur in germinal center B cells in response to signals from Tfh cells. Mutations that result in increased affinity of antibodies for antigen impart a selective survival advantage to the B cells producing those antibodies and lead to affinity maturation of a humoral immune response.

Somatic recombination The process of DNA recombination by which the functional genes encoding the variable regions of antigen receptors are formed during lymphocyte development. A relatively limited set of inherited, or germline, DNA sequences that are initially separated from one another are brought together by enzymatic deletion of intervening sequences and re-ligation. This process occurs only in developing B or T lymphocytes and is mediated by RAG-1 and RAG-2 proteins. This process is also called **V(D)J recombination.**

Specificity A cardinal feature of the adaptive immune system, namely, that immune responses are directed toward and able to distinguish between distinct antigens and small parts of macromolecular antigens. This fine specificity is attributed to lymphocyte antigen receptors that may bind to one molecule but not to another, even closely related, molecule.

Spleen A secondary lymphoid organ in the left upper quadrant of the abdomen. The spleen is the major site of adaptive immune responses to blood-borne antigens. The red pulp of the spleen is composed of blood-filled vascular sinusoids lined by active phagocytes that ingest opsonized antigens and damaged red blood cells. The white pulp of the spleen contains lymphocytes and lymphoid follicles where B cells are activated.

SRC-family kinases A family of protein tyrosine kinases, homologous to the SRC tyrosine kinase, which in immune cells, initiate signaling downstream of immune receptors by phosphorylating tyrosine residues on ITAM motifs. LCK is a prominent SRC-family kinase in T cells and LYN in B cells.

SRC homology 2 (SH2) domain A three-dimensional domain structure of approximately 100 amino acid residues present in many signaling proteins that permits specific noncovalent interactions with other proteins by binding to phosphotyrosines. Each SH2 domain has a unique binding specificity that is determined by the amino acid residues adjacent to the phosphotyrosine on the target protein. Several proteins involved in early signaling events in T and B lymphocytes interact with one another through SH2 domains.

SRC homology 3 (SH3) domain A three-dimensional domain structure of approximately 60 amino acid residues present in many signaling proteins that mediates protein-protein binding. SH3 domains bind to proline residues and function cooperatively with the SH2 domains of the same protein. For instance, SOS, the guanine nucleotide exchange factor for Ras, contains both SH2 and SH3 domains, and both are involved in SOS binding to the adaptor protein GRB2.

Stem cell An undifferentiated cell that divides continuously and gives rise to additional stem cells and to cells of multiple different lineages. For example, all blood cells arise from a common hematopoietic stem cell.

STING (Stimulator of IFN Genes) An adaptor protein located in the endoplasmic reticulum membrane, which is used by several cytoplasmic DNA sensor molecules to transduce signals that activate the IRF3 transcription factor, leading to type I IFN gene expression.

Superantigens Microbial proteins that bind to and activate all of the T cells in an individual that express a particular set or family of Vβ TCR genes. Superantigens are presented to T cells by binding to nonpolymorphic regions of class II MHC molecules on APCs, and they interact with conserved regions of TCR Vβ domains. Several staphylococcal enterotoxins are superantigens. Their importance lies in their ability to activate many T cells, which results in large amounts of cytokine production and a clinical syndrome that is similar to septic shock.

Suppressor T cells T cells that block the activation and function of other T lymphocytes. These cells were described in the 1970s, but because it has been difficult to clearly define suppressor T cells or their mode of action, the term is no longer used.

Surrogate light chains Two nonvariable proteins that associate with Ig μ heavy chains in pre-B cells to form the pre-BCR. The two surrogate light chain proteins include the V pre-B protein, which is homologous to a light-chain V domain, and λ5, which is covalently attached to the μ heavy chain by a disulfide bond.

Switch recombination The molecular mechanism underlying Ig isotype switching in which a rearranged VDJ gene segment in an antibody-producing B cell recombines with a downstream C gene and the intervening C genes are deleted. DNA recombination events in switch recombination are triggered by CD40 and cytokines and involve nucleotide sequences called switch regions located in the introns at the 5′ end of each C_H locus.

SYK A cytoplasmic protein tyrosine kinase, similar to ZAP70 in T cells, that is critical for early signaling steps in antigen-induced B cell activation. SYK binds to phosphorylated tyrosines in the cytoplasmic tails of the Igα and Igβ chains of the BCR complex and in turn phosphorylates adaptor proteins that recruit other components of the signaling cascade.

Syngeneic Genetically identical. All animals of an inbred strain and monozygotic twins are syngeneic.

Syngeneic graft A graft from a donor who is genetically identical to the recipient. Syngeneic grafts are not rejected.

Synthetic vaccine Vaccines composed of recombinant DNA-derived antigens. Synthetic vaccines for hepatitis B virus and herpes simplex virus are now in use.

Systemic inflammatory response syndrome (SIRS) The systemic changes observed in patients who have disseminated bacterial infections and other conditions that induce widespread inflammation, such as burns. In its mild form, SIRS consists of neutrophilia, fever, and a rise in acute-phase reactants in the plasma. These changes are stimulated by bacterial products such as lipopolysaccharide and are mediated by cytokines of the innate immune system. In severe cases, SIRS may include disseminated intravascular coagulation, acute respiratory distress syndrome, and shock.

Systemic lupus erythematosus (SLE) A chronic systemic autoimmune disease that affects predominantly women and is characterized by rashes, arthritis, glomerulonephritis, hemolytic anemia, thrombocytopenia, and central nervous system involvement. Many different autoantibodies are found in patients with SLE, particularly anti-DNA antibodies. Many of the manifestations of SLE are due to the formation of immune complexes composed of autoantibodies and their specific antigens, with deposition of these complexes in small blood vessels in various tissues. The underlying mechanism for the breakdown of self-tolerance in SLE is not understood.

T

T cell exhaustion A dysfunctional state of T cells, especially CD8$^+$ CTLs, that is induced by persistent antigen stimulation in the setting of chronic viral infection or cancer and is characterized by decreased proliferation, reduced cytokine production, and poor cytotoxic activity, leading to an inability to clear infections or tumors. Exhaustion results from a block in T cell differentiation caused in part by increased expression of inhibitory molecules such as PD-1.

T cell receptor (TCR) The clonally distributed antigen receptor on CD4$^+$ and CD8$^+$ T lymphocytes that recognizes complexes of foreign peptides bound to self MHC molecules on the surface of APCs. The most common form of TCR is composed of a heterodimer of two disulfide-linked transmembrane polypeptide chains, designated α and β, each containing one N-terminal Ig-like variable (V) domain, one Ig-like constant (C) domain, a hydrophobic transmembrane region, and a short cytoplasmic region. Another less common type of TCR, composed of γ and δ chains, is found on a small subset of T cells and recognizes different forms of antigen.

T cell receptor (TCR) transgenic mouse A mouse in a genetically engineered strain that expresses transgene-encoded functional TCR α and β genes encoding a TCR of a single defined specificity. Because of allelic exclusion of endogenous TCR genes, most or all of the T cells in a TCR transgenic mouse have the same antigen specificity, which is a useful property for various research purposes.

T follicular helper (Tfh) cells A subset of $CD4^+$ helper T cells present within lymphoid follicles that are critical in providing signals to B cells in the germinal center reaction that stimulate somatic hypermutation, isotype switching, and the generation of memory B cells and long-lived plasma cells. Tfh cells express CXCR5, ICOS, IL-21, and BCL-6.

T lymphocyte The key component of cell-mediated immune responses in the adaptive immune system. T lymphocytes mature in the thymus, circulate in the blood, populate secondary lymphoid tissues, and are recruited to peripheral sites of antigen exposure. They express antigen receptors (TCRs) that recognize peptide fragments of foreign proteins bound to self MHC molecules. Functional subsets of T lymphocytes include $CD4^+$ helper T cells and $CD8^+$ CTLs.

Tacrolimus An immunosuppressive drug (also known as FK506) used to prevent allograft rejection that functions by blocking T cell cytokine gene transcription, similar to cyclosporine. Tacrolimus binds to a cytosolic protein called FK506-binding protein, and the resulting complex binds to calcineurin, thereby inhibiting activation and nuclear translocation of the transcription factor NFAT.

T-BET A T-box family transcription factor that promotes the differentiation of Th1 cells from naive T cells.

T-dependent antigen An antigen that requires both B cells and helper T cells to stimulate an antibody response. T-dependent antigens are protein antigens that contain some epitopes recognized by T cells and other epitopes recognized by B cells. Helper T cells produce cytokines and cell surface molecules that stimulate B cell growth and differentiation into antibody-secreting cells. Humoral immune responses to T-dependent antigens are characterized by isotype switching, affinity maturation, long-lived plasma cells, and memory.

Tertiary lymphoid organ A collection of lymphocytes and APCs cells organized into B cell follicles and T cell zones that develop in sites of chronic immune-mediated inflammation, such as the joint synovium of rheumatoid arthritis patients.

Th1 cells A subset of $CD4^+$ helper T cells that secrete a particular set of cytokines, including IFN-γ, and whose principal function is to stimulate phagocyte-mediated defense against infections, especially with intracellular microbes.

Th2 cells A subset of $CD4^+$ helper T cells that secrete a particular set of cytokines, including IL-4, IL-5, and IL-3, and whose principal function is to stimulate IgE and eosinophil/mast cell–mediated immune reactions.

Th17 cells A subset of $CD4^+$ helper T cells that secrete a particular set of cytokines, including IL-17 and IL-22, that are protective against bacterial and fungal infections and also mediate inflammatory reactions in autoimmune and other inflammatory diseases.

Thymic epithelial cells Epithelial cells abundant in the cortical and medullary stroma of the thymus that play a critical role in T cell development. In the process of positive selection, maturing T cells that weakly recognize self peptides bound to MHC molecules on the surface of thymic epithelial cells are rescued from programmed cell death.

Thymocyte A precursor of a mature T lymphocyte present in the thymus.

Thymus A bilobed organ situated in the anterior mediastinum that is the site of maturation of T lymphocytes from bone marrow–derived precursors. The thymus is divided into an outer cortex and an inner medulla and contains stromal thymic epithelial cells, macrophages, dendritic cells, and numerous T cell precursors (thymocytes) at various stages of maturation.

T-independent antigen Nonprotein antigens, such as polysaccharides and lipids, which can stimulate antibody responses without a requirement for antigen-specific helper T lymphocytes. T-independent antigens usually contain multiple identical epitopes that can cross-link membrane Ig on B cells and thereby activate the cells. Humoral immune responses to T-independent antigens show relatively little heavy chain isotype switching or affinity maturation, two processes that require signals from helper T cells.

Tissue typing The determination of the particular MHC alleles expressed by an individual for the purpose of matching allograft donors and recipients. Tissue typing, also called HLA typing, is usually done by molecular (PCR-based) sequencing of HLA alleles or by serologic methods (lysis of an individual's cells by panels of anti-HLA antibodies).

TNF receptor-associated factors (TRAFs) A family of adaptor molecules that interact with the cytoplasmic domains of various receptors in the TNF receptor family, including TNF-RII, lymphotoxin (LT)-β receptor, and CD40. Each of these receptors contains a cytoplasmic motif that binds different TRAFs, which in turn engage other signaling molecules, leading to activation of the transcription factors AP1 and NF-κB.

Tolerance Unresponsiveness of the adaptive immune system to antigens, as a result of inactivation or death of antigen-specific lymphocytes, induced by exposure to the antigens. Tolerance to self antigens is a normal feature of the adaptive immune system, but tolerance to foreign antigens may be induced under certain conditions of antigen exposure.

Tolerogen An antigen that induces immunologic tolerance, in contrast to an immunogen, which induces an immune response. Many antigens can be either tolerogens or immunogens, depending on how they are administered. Tolerogenic forms of antigens include large doses of proteins administered without adjuvants and orally administered antigens.

Toll-like receptors A family of pattern recognition receptors of the innate immune system that are expressed by many cell types and recognize microbial structures, such as flagellin, lipopolysaccharide, peptidoglycan, double-stranded RNA, and CpG DNA. TLRs transduce signals that lead to the expression of inflammatory and antiviral genes. There are 10 human TLRs, 7 of which are expressed on the plasma membrane of cells and 3 are located in endosomal membranes.

Tonsils Partially encapsulated secondary lymphoid tissues located beneath barrier epithelium in the nasopharynx and oropharynx, including adenoids (pharyngeal tonsils), palatine tonsils, and lingual tonsils. Tonsils are sites of initiation of adaptive immune responses to microbes in the upper respiratory and alimentary tracts.

Toxic shock syndrome An acute illness characterized by shock, skin exfoliation, conjunctivitis, and diarrhea that is associated with tampon use and caused by a *Staphylococcus aureus* superantigen, which is a polyclonal activator of all T cells expressing TCRs using a particular subgroup of Vβ genes.

Transfusion Transplantation of circulating blood cells, platelets, or plasma from one individual to another. Transfusions are performed to treat blood loss from hemorrhage or to treat a deficiency in one or more blood cell types resulting from inadequate production or excess destruction.

Transfusion reactions An immunologic reaction against transfused blood products, usually mediated by preformed antibodies in the recipient that bind to donor blood cell antigens, such as ABO blood group antigens or histocompatibility antigens. Transfusion reactions can lead to intravascular lysis of red blood cells and, in severe cases, kidney damage, fever, shock, and disseminated intravascular coagulation.

Transgenic mouse A mouse that expresses an exogenous gene that has been introduced into the genome by injection of a specific DNA sequence into the pro-nuclei of fertilized mouse eggs. Transgenes insert randomly at chromosomal break points and are subsequently inherited as simple Mendelian traits. By the design of transgenes with tissue-specific regulatory sequences, mice can be produced that express a particular gene only in certain tissues. Transgenic mice are used extensively in immunology research to study the functions of various cytokines, cell surface molecules, and intracellular signaling molecules.

Transplantation The process of transferring cells, tissues, or organs (i.e., grafts) from one individual to another or from one site to another in the same individual. Transplantation is used to treat a variety of diseases in which there is a functional disorder of a tissue or organ. The major barrier to successful transplantation between individuals is immunologic reaction (rejection) to the transplanted graft.

Transporter associated with antigen processing (TAP) An ATP-dependent peptide transporter that mediates the active transport of peptides from the cytosol to the site of assembly of class I MHC molecules inside the endoplasmic reticulum. TAP is a heterodimeric molecule composed of TAP-1 and TAP-2 polypeptides, both encoded by genes in the MHC. Because peptides are required for stable assembly of class I MHC molecules, TAP-deficient animals express few cell surface class I MHC molecules, which results in diminished development and activation of $CD8^+$ T cells.

Tuft cell A specialized intestinal epithelial cell type that is involved in enhancing mucus secretion in response to helminth infection. Tuft cells are activated by helminths to secrete IL-25, which stimulates ILC2s to secrete IL-13, which in turn promotes formation of mucus-secreting goblet cells.

Tumor immunity Protection against the development or progression of tumors by the immune system. Although immune responses to naturally occurring tumors can frequently be demonstrated, tumors often escape these responses. New therapies that target T cell inhibitory molecules, such as PD-1, are proving effective in enhancing effective T cell–mediated antitumor immunity.

Tumor-infiltrating lymphocytes (TILs) Lymphocytes isolated from the inflammatory infiltrates present in and around surgical resection samples of solid tumors that are enriched with tumor-specific CTLs and NK cells. In an experimental mode of cancer treatment, TILs are grown in vitro in the presence of high doses of IL-2 and are then adoptively transferred back into patients with the tumor.

Tumor necrosis factor receptor superfamily (TNFRSF) A large family of structurally homologous transmembrane proteins that bind TNFSF proteins and generate signals that regulate proliferation, differentiation, apoptosis, and inflammatory gene expression (*see* Appendix II).

Tumor necrosis factor superfamily (TNFSF) A large family of structurally homologous transmembrane proteins that regulate diverse functions in responding cells, including proliferation, differentiation, apoptosis, and inflammatory gene expression. TNFSF members typically form homotrimers, either within the plasma membrane or after proteolytic release from the membrane, and bind to homotrimeric TNF receptor superfamily (TNFRSF) molecules, which then initiate a variety of signaling pathways (*see* Appendix II).

Tumor-specific antigen An antigen whose expression is restricted to a particular tumor and is not expressed by normal cells. Tumor-specific antigens may serve as target antigens for antitumor immune responses.

Two-signal hypothesis A now-proved hypothesis that states that the activation of lymphocytes requires two distinct signals, the first being antigen and the second either microbial products or components of innate immune responses to microbes. The requirement for antigen (so-called signal 1) ensures that the ensuing immune response is specific. The requirement for additional stimuli triggered by microbes or innate immune reactions (signal 2) ensures that immune responses are induced when they are needed, that is, against microbes and other noxious substances and not against harmless substances, including self antigens. Signal 2 is referred to as costimulation and is often mediated by membrane molecules on professional APCs, such as B7 proteins.

Type 1 diabetes A disease characterized by a lack of insulin that leads to various metabolic and vascular abnormalities. The insulin deficiency results from autoimmune destruction of the insulin-producing β cells of the islets of Langerhans in the pancreas, usually during childhood. $CD4^+$ and $CD8^+$ T cells, antibodies, and cytokines have been implicated in the islet cell damage. Also called insulin-dependent diabetes mellitus.

Type 1 immune response An immune response dominated by ILC1s and Th1 cells and the IFN-γ they produce.

Type 2 immune response An immune response typical of allergic diseases, dominated by ILC2s and Th2 cells and the cytokines they produce, including IL-4, IL-5, and IL-13. Type 2 responses may begin with epithelial injury and secretion of the cytokines TSLP, IL-25, and IL-33.

Type 3 immune response An immune response dominated by ILC3s and Th17 cells and the cytokines they produce, including IL-17 and IL-22.

U

Ubiquitination Covalent linkage of one or several copies of a small polypeptide called ubiquitin to a protein. Ubiquitination frequently serves to target proteins for proteolytic degradation mainly in proteasomes, a critical step in the class I MHC pathway of antigen processing and presentation.

Uracil N-glycosylase (UNG) An enzyme that removes uracil residues from DNA, leaving an abasic site. UNG is a key participant in isotype switching, and homozygous UNG mutations result in a hyper-IgM syndrome.

Urticaria Localized transient itchy swelling and redness of the skin caused by leakage of fluid and plasma proteins from small vessels into the upper dermis during an immediate hypersensitivity reaction.

V

V gene segments A DNA sequence that encodes most of the variable domain of an Ig heavy chain or light chain or a TCR α, β, γ, or δ chain. Each antigen receptor locus contains many different V gene segments, any one of which may recombine with downstream D or J segments during lymphocyte maturation to form functional antigen receptor V genes.

V(D)J recombinase The complex of RAG1 and RAG2 proteins that catalyzes lymphocyte antigen receptor gene recombination.

Vaccine A preparation of microbial antigen, often combined with adjuvants, that is administered to individuals to induce protective immunity against microbial infections. The antigen may be in the form of live but avirulent microorganisms, killed microorganisms, purified macromolecular components of a microorganism, or a plasmid that contains a complementary DNA encoding a microbial antigen.

Variable region The extracellular, N-terminal region of an Ig heavy or light chain or a TCR α, β, γ, or δ chain that contains variable amino acid sequences that differ between every clone of lymphocytes and that are responsible for the specificity for antigen. The antigen-binding variable sequences are localized to extended loop structures or hypervariable segments.

Vasoactive amines Low-molecular-weight nonlipid compounds, such as histamine, that all have an amine group, are stored in and released from the cytoplasmic granules of mast cells and mediate many of the biologic effects of immediate hypersensitivity (allergic) reactions. (Also called biogenic amines.)

Virus An obligate intracellular microorganism or infectious particle that consists of a simple nucleic acid genome packaged in a protein capsid, sometimes surrounded by a membrane envelope. Many pathogenic animal viruses cause a wide range of diseases. Humoral immune responses to viruses can be effective in blocking infection of cells, and NK cells and CTLs are necessary to kill cells already infected.

W

Western blot An immunologic technique to determine the presence of a protein in a biologic sample. The method involves separation of proteins in the sample by electrophoresis, transfer of the protein array from the electrophoresis gel to a support membrane by capillary action (blotting) and detection of the protein by binding of an enzymatically or radioactively labeled antibody specific for that protein.

Wheal-and-flare reaction Local swelling and redness in the skin at a site of an immediate hypersensitivity reaction. The wheal reflects increased vascular permeability, and the flare results from increased local blood flow, both changes resulting from mediators such as histamine released from activated dermal mast cells.

White pulp The part of the spleen that is composed predominantly of lymphocytes, arranged in periarteriolar lymphoid sheaths and follicles; and other leukocytes, mainly macrophages and DCs. The remainder of the spleen contains sinusoids lined with phagocytic cells and filled with blood, called the **red pulp.**

Wiskott-Aldrich syndrome An X-linked disease characterized by eczema, thrombocytopenia (reduced blood platelets), and immunodeficiency manifested as susceptibility to bacterial infections. The defective gene encodes a cytosolic protein involved in signaling cascades and regulation of the actin cytoskeleton.

X

XBP-1 A transcription factor that is required for the unfolded protein response and for the development of plasma cells.

Xenoantigen An antigen on a graft from another species.

Xenograft (xenogeneic graft) An organ or tissue graft derived from a species different from the recipient. Transplantation of xenogeneic grafts (e.g., from a pig) to humans is not yet feasible because of problems related to immunologic rejection.

Xenoreactive Describing a T cell or antibody that recognizes and responds to an antigen on a graft from another species (a xenoantigen). The T cell may recognize an intact xenogeneic MHC molecule or a peptide derived from a xenogeneic protein bound to a self MHC molecule.

X-linked agammaglobulinemia An immunodeficiency disease, also called Bruton's agammaglobulinemia, characterized by a block in early B cell maturation and an absence of serum Ig. Patients suffer from pyogenic bacterial infections. The disease is caused by mutations or deletions in the gene encoding BTK, an enzyme involved in signal transduction in developing B cells.

X-linked hyper-IgM syndrome A rare immunodeficiency disease caused by mutations in the CD40 ligand gene and characterized by failure of B cell heavy chain isotype switching and defective cell-mediated immunity. Patients suffer from pyogenic bacterial and intracellular infections.

Z

ζ **Chain** A transmembrane protein expressed in T cells as part of the TCR complex that contains ITAMs in its cytoplasmic tail and binds the ZAP70 protein tyrosine kinase during T cell activation.

Zeta-associated protein of 70 kD (ZAP70) A cytoplasmic protein tyrosine kinase, similar to SYK in B cells, that is critical for early signaling steps in antigen-induced T cell activation. ZAP70 binds to phosphorylated tyrosines in the cytoplasmic tails of the ζ chain and CD3 chains of the TCR complex and in turn phosphorylates adaptor proteins that recruit other components of the signaling cascade.

Appendix I

Principal Features of Selected CD Molecules

The following list includes selected CD molecules that are referred to in the text. Many cytokines and cytokine receptors have been assigned CD numbers, but we refer to these by the more descriptive cytokine designation, and these are listed in Appendix II. A complete and up-to-date listing of CD molecules may be found at http://www.hcdm.org.

CD Number (Other Names)	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD1a–d	36–44 kD; class I MHC-like Ig superfamily; β_2-microglobulin associated	Thymocytes, DCs	Presentation of nonpeptide (lipid and glycolipid) antigens to iNKT cells
CD1e	44 kD; class I MHC-like; β2-microglobulin associated	DCs	Same as CD1a
CD2 (LFA-2)	50 kD; Ig superfamily	T cells, NK cells	Adhesion molecule (binds CD58); T cell activation; CTL- and NK cell–mediated lysis
CD3γ	25–28 kD; associated with CD3δ and CD3ε in TCR complex; Ig superfamily; ITAM in cytoplasmic tail	T cells	Cell surface expression of and signal transduction by the T cell antigen receptor
CD3δ	20 kD; associated with CD3γ and CD3ε in TCR complex; Ig superfamily; ITAM in cytoplasmic tail	T cells	Cell surface expression of and signal transduction by the T cell antigen receptor
CD3ε	23 kD; associated with CD3δ and CD3γ in TCR complex; Ig superfamily; ITAM in cytoplasmic tail	T cells	Cell surface expression of and signal transduction by the T cell antigen receptor
CD4	55 kD; Ig superfamily	Class II MHC–restricted T cells; some macrophages	Coreceptor in class II MHC-restricted antigen-induced T cell activation (binds to class II MHC molecules); thymocyte development; receptor for HIV
CD5	67 kD; scavenger receptor family	T cells; B-1 B cell subset	Signaling molecule
CD8α	34 kD; expressed as a homodimer or heterodimer with CD8β	Class I MHC–restricted T cells; subset of DCs	Coreceptor in class I MHC–restricted antigen-induced T cell activation (binds to class I MHC molecules); thymocyte development

Table Continued

CD Number (Other Names)	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD8β	34 kD; expressed as a heterodimer with CD8α; Ig superfamily	Class I MHC–restricted T cells	Same as CD8α
CD10	100 kD; type II membrane protein	Immature and some mature B cells; lymphoid progenitors, granulocytes	Metalloproteinase; unknown function in the immune system
CD11a (LFA-1 α chain)	180 kD; noncovalently linked to CD18 to form LFA-1 integrin	Leukocytes	Cell-cell adhesion; binds to ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50)
CD11b (Mac-1; CR3 α chain)	165 kD; noncovalently linked to CD18 to form MAC-1 integrin	Granulocytes, monocytes, macrophages, DCs, NK cells	Phagocytosis of iC3b-coated particles; neutrophil and monocyte adhesion to endothelium (binds CD54) and extracellular matrix proteins
CD11c (p150,95; CR4α chain; integrin αx)	145 kD; noncovalently linked to CD18 to form p150,95 integrin	DCs, monocytes, macrophages, granulocytes, NK cells	Similar functions as CD11b
CD14	53 kD; GPI linked	DCs, monocytes, macrophages, granulocytes	Binds complex of LPS and LPS-binding protein DCs and displays LPS to TLR4; required for LPS-induced macrophage activation
CD16a (FcγRIIIA)	50–70 kD; transmembrane protein; Ig superfamily	NK cells, macrophages	Binds Fc region of IgG; phagocytosis and antibody-dependent cellular cytotoxicity
CD16b (FcγRIIIB)	50–70 kD; GPI linked; Ig superfamily	Neutrophils	Binds Fc region of IgG; synergy with FcγRII in immune complex–mediated neutrophil activation
CD18	95 kD; noncovalently linked to CD11a, CD11b, or CD11c to form β_2 integrins	Leukocytes	See CD11a, CD11b, CD11c
CD19	95 kD; Ig superfamily	Most B cells	B cell activation; forms a coreceptor complex with CD21 and CD81 that delivers signals that synergize with signals from B cell antigen receptor complex
CD20	35–37 kD; Membrane-spanning 4A (MS4A)) family	B cells	Unknown; target for B cell–depleting antibody
CD21 (CR2; C3d receptor)	145 kD; regulators of complement activation (RCA) family	Mature B cells, follicular DCs	Receptor for complement fragment C3d; forms a coreceptor complex with CD19 and CD81 that delivers activating signals in B cells; receptor for Epstein-Barr virus
CD22 (Siglec-2)	130–140 kD; Ig superfamily; sialoadhesin family; ITIM in cytoplasmic tail	B cells	Regulation of B cell activation; adhesion molecule
CD23 (FcεRII)	45 kD; C-type lectin	Activated B cells, monocytes, macrophages	Low-affinity Fcε receptor, induced by IL-4; function unknown
CD25 (IL-2 receptor α chain)	55 kD; noncovalently associated with IL-2Rβ (CD122) and IL-2Rγ (CD132) chains to form a high-affinity IL-2 receptor	Activated T and B cells, regulatory T cells (Treg)	Binds IL-2 and promotes responses to low concentrations of IL-2
CD28	Homodimer of 44-kD chains; Ig superfamily	T cells (all CD4$^+$ and >50% of CD8$^+$ cells in humans; all mature T cells in mice)	Receptor on T cells for costimulatory molecules CD80 (B7-1) and CD86 (B7-2)

Table Continued

CD Number (Other Names)	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD29	130 kD; noncovalently linked to CD49a–d chains to form VLA (β_1) integrins	T cells, B cells, monocytes, granulocytes	Leukocyte adhesion to extracellular matrix proteins and endothelium (see CD49)
CD30 (TNFRSF8)	120 kD; TNFR superfamily	Activated T and B cells; NK cells, monocytes, Reed-Sternberg cells in Hodgkin's disease	Unknown
CD31 (platelet/endothelial cell adhesion molecule 1 [PECAM-1])	130–140 kD; Ig superfamily	Platelets, monocytes, granulocytes, B cells, endothelial cells	Adhesion molecule involved in leukocyte transmigration through endothelium
CD32 (FcγRII)	40 kD; Ig superfamily; ITIM in cytoplasmic tail; A, B, and C forms are products of different but homologous genes	B cells, macrophages, DCs, granulocytes	Fc receptor for aggregated IgG; acts as inhibitory receptor that blocks activation signals in B cells and other cells
CD34	105–120 kD; sialomucin	Precursors of hematopoietic cells; endothelial cells in high endothelial venules	Unknown
CD35 (type 1 complement receptor, CR1)	190–285 kD (four products of polymorphic alleles); regulator of complement activation family	Granulocytes, monocytes, erythrocytes, B cells, follicular DCs, some T cells	Binds C3b and C4b; promotes phagocytosis of C3b- or C4b-coated particles and immune complexes; regulates complement activation
CD36	85–90 kD	Platelets, monocytes, macrophages, endothelial cells	Scavenger receptor for oxidized low-density lipoprotein; platelet adhesion; phagocytosis of apoptotic cells
CD40	Homodimer of 44- to 48-kD chains; TNFR superfamily	B cells, macrophages, DCs, endothelial cells	Binds CD154 (CD40L); role in T cell–mediated activation of B cells, macrophages, and DCs
CD43 (Sialophorin, leukosialin)	95–135 kD; sialomucin	Leukocytes (except circulating B cells)	Unknown
CD44	80 to >100 kD, highly glycosylated	Leukocytes, erythrocytes	Binds hyaluronan; involved in leukocyte adhesion to endothelial cells and extracellular matrix
CD45 (leukocyte common antigen [LCA], B220)	Multiple isoforms, 180–220 kD (see CD45R); protein tyrosine phosphatase receptor family; fibronectin type III family	Hematopoietic cells	Tyrosine phosphatase that regulates T and B cell activation
CD45R	CD45RO:180 kD; CD45RA: 220 kD; CD45RB: 190, 205, and 220 kD isoforms	CD45RO: memory T cells; subset of B cells, monocytes, macrophages CD45RA: naive T cells, B cells, monocytes CD45RB: B cells, subset of T cells	See CD45
CD46 (membrane cofactor protein [MCP])	52–58 kD; regulators of complement activation family	Leukocytes, epithelial cells, fibroblasts	Regulation of complement activation
CD47	47–52 kD; Ig superfamily	All hematopoietic cells, epithelial cells, endothelial cells, fibroblasts	Leukocyte adhesion, migration, activation; "Don't eat me" signal to phagocytes
CD49d	150 kD; noncovalently linked to CD29 to form VLA-4 ($\alpha4\beta1$ integrin)	T cells, monocytes, B cells, NK cells, eosinophils, DCs, thymocytes	Leukocyte adhesion to endothelium and extracellular matrix; binds to VCAM-1 and MAdCAM-1; binds fibronectin and collagens

Table Continued

CD Number (Other Names)	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD52	6.6kD; GPI-linked protein	B cells, T cells, monocytes, DCs, sperm cells	Binds SIGLEC10; negative charges may prevent cell adhesion; target for immunotherapy of lymphomas and T cell depletion
CD54 (ICAM-1)	75–114 kD; Ig superfamily	T cells, B cells, monocytes, endothelial cells (cytokine inducible)	Cell-cell adhesion; ligand for CD11aCD18 (LFA-1) and CD11bCD18 (Mac-1); receptor for rhinovirus
CD55 (decay-accelerating factor [DAF])	55–70 kD; GPI linked; regulators of complement activation family	Broad	Regulation of complement activation
CD58 (leukocyte function–associated antigen 3 [LFA-3])	55–70 kD; GPI-linked or integral membrane protein	Broad	Leukocyte adhesion; binds CD2
CD59	18–20 kD; GPI linked	Broad	Binds C9; inhibits formation of complement membrane attack complex
CD62E (E-selectin)	115 kD; selectin family	Endothelial cells	Leukocyte-endothelial adhesion; homing of leukocytes to inflamed tissues
CD62L (L-selectin)	74–95 kD; selectin family	B cells, T cells, monocytes, granulocytes, some NK cells	Leukocyte-endothelial adhesion; homing of naive T cells to lymph nodes
CD62P (P-selectin)	140 kD; selectin family	Platelets, endothelial cells (present in granules, translocated to cell surface on activation)	Leukocyte adhesion to endothelium, platelets; binds CD162 (PSGL-1)
CD64 (FcγRI)	72 kD; Ig superfamily; noncovalently associated with the FcR common γ chain	Monocytes, macrophages, activated neutrophils	High-affinity Fcγ receptor; role in phagocytosis, ADCC, macrophage activation
CD66e (carcinoembryonic antigen [CEA])	180–220 kD; Ig superfamily; CEA family	Colonic and other epithelial cells	Adhesion of epithelial cells?; clinical marker of carcinoma burden
CD69	23 kD; C-type lectin	Activated B cells, T cells, NK cells, neutrophils	Binds to and impairs surface expression of S1PR1, thereby promoting retention of activated lymphocytes in lymphoid organs and nonlymphoid tissues
CD74 (class II MHC invariant chain [I_i])	33-, 35-, and 41-kD isoforms	B cells, DCs, macrophages	Binds to and directs intracellular sorting of newly synthesized class II MHC molecules
CD79a (Igα)	33, 45 kD; forms dimer with CD79β; Ig superfamily; ITAM in cytoplasmic tail	Mature B cells; pre-B cells	Required for cell surface expression of and signal transduction by the B cell and pre-B receptor complex
CD79b (Igβ)	37–39 kD; forms dimer with CD79α; Ig superfamily; ITAM in cytoplasmic tail	Mature B cells; pre-B cells	Required for cell surface expression of and signal transduction by the B cell and pre-B receptor complex
CD80 (B7-1)	60 kD; Ig superfamily	DCs, activated B cells and macrophages	Costimulator for T lymphocyte activation; ligand for CD28 and CD152 (CTLA-4)
CD81 (target for anti-proliferative antigen 1 [TAPA-1], TSPAN-28)	26 kD; tetraspanin family	T cells, B cells, NK cells, DCs, thymocytes, endothelial cells	B cell activation; forms a coreceptor complex with CD19 and CD21 that delivers signals that synergize with signals from the B cell antigen receptor complex

Table Continued

CD Number (Other Names)	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD86 (B7-2)	80 kD; Ig superfamily	B cells, monocytes; DCs; some T cells	Costimulator for T lymphocyte activation; ligand for CD28 and CD152 (CTLA-4)
CD88 (C5a receptor)	43 kD; G protein–coupled, seven membrane-spanning receptor family	Granulocytes, monocytes, DCs, mast cells	Receptor for C5a complement fragment; role in complement-induced inflammation
CD89 (Fcα receptor [FcαR])	55–75 kD; Ig superfamily; noncovalently associated with the common FcRγ chain	Granulocytes, monocytes, macrophages, T cell subset, B cell subset	Binds IgA and C reactive protein; function not clear
CD90 (Thy-1)	25–35 kD; GPI linked; Ig superfamily	Thymocytes, peripheral T cells (mice), CD34$^+$ hematopoietic progenitor cells, neurons	Marker for T cells; unknown function
CD94	43 kD; C-type lectin; on NK cells, covalently assembles with other C-type lectin molecules (NKG2)	NK cells; subset of CD8$^+$ T cells	CD94/NKG2 complex functions as an NK cell inhibitory receptor; binds HLA-E class I MHC molecules
CD95 (FAS)	Homotrimer of 45-kD chains; TNFR superfamily	Broad	Binds FAS ligand; delivers signals leading to apoptotic death
CD102 (ICAM-2)	55–65 kD; Ig superfamily	Endothelial cells, lymphocytes, monocytes, platelets	Ligand for CD11aCD18 (LFA-1); cell-cell adhesion
CD103 (α_E integrin subunit)	Dimer of 150- and 25-kD subunits; noncovalently linked to β_7 integrin subunit to form $\alpha_E\beta_7$ integrin	Intraepithelial lymphocytes, other cell types	Role in T cell homing to and retention in mucosa; binds E-cadherin
CD106 (vascular cell adhesion molecule 1 [VCAM-1])	100–110 kD; Ig superfamily	Endothelial cells, macrophages, FDCs, marrow stromal cells	Adhesion of cells to endothelium; receptor for CD49dCD29 (VLA-4) integrin; role in lymphocyte trafficking, activation
CD134 (OX40, TNFRSF4)	29 kD; TNFR superfamily	Activated T cells	Receptor for T cell CD252; T cell costimulation
CD141 (BDCA-3, CLEC9A, thrombomodulin)	60 kD; EGF-like domains	Cross-presenting DCs, monocytes, endothelial cells	Binds thrombin and prevents blood coagulation
CD150 (Signaling lymphocyte activation molecule [SLAM])	37 kD; Ig superfamily	Thymocytes, activated lymphocytes, DCs, endothelial cells	Activation of B and T cells
CD152 (cytotoxic T lymphocyte–associated protein 4 [CTLA-4])	33, 50 kD; Ig superfamily	Activated T lymphocytes, regulatory T cells	Mediates suppressive function of regulatory T cells; inhibits T cell responses; binds CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells
CD154 (CD40 ligand [CD40L])	Homotrimer of 32- to 39-kD chains; TNF superfamily	Activated CD4$^+$ T cells	Activation of B cells, macrophages, and endothelial cells; ligand for CD40
CD158 (killer cell Ig-like receptor [KIR])	50, 58 kD; Ig superfamily; KIR family; ITIMs or ITAMs in cytoplasmic tail	NK cells, T cell subset	Inhibition or activation of NK cells on interaction with appropriate class I HLA molecules
CD159a (NKG2A)	43 kD; C-type lectin; ITIM in cytoplasmic tail; forms heterodimer with CD94	NK cells, T cell subset	Inhibition or activation of NK cells on interaction with class I HLA molecules
CD159c (NKG2C)	40 kD; C-type lectin; forms heterodimer with CD94	NK cells	Activation of NK cells on interaction with the appropriate class I HLA molecules

Table Continued

CD Number (Other Names)	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD162 (P-selectin glycoprotein ligand 1 [PSGL-1])	Homodimer of 120-kD chains; sialomucin	T cells, monocytes, granulocytes, some B cells	Ligand for selectins (CD62P, CD62L); adhesion of leukocytes to endothelium
CD178 (FAS ligand [FASL])	Homotrimer of 31-kD subunits; TNF superfamily	Activated T cells	Ligand for CD95 (FAS); triggers apoptotic death
CD206 (mannose receptor)	166 kD; C-type lectin	Macrophages	Binds terminal mannose-containing glycoproteins on pathogens; mediates macrophage endocytosis of glycoproteins and phagocytosis of bacteria, fungi, and other pathogens
CD223 (Lymphocyte-activation gene 3 [LAG3])	57.4 kD; Ig superfamily;	T cells, NK cells, B cells, plasmacytoid DCs	Binds class II MHC and galectin-3; negatively regulates T cell activation
CD244 (2B4)	41 kD; Ig superfamily; CD2/CD48/CD58 family; SLAM family	NK cells, CD8 T cells, γδ T cells	Receptor for CD148; modulates NK cell cytolytic activity
CD247 (TCR ζ chain)	18 kD; ITAMs in cytoplasmic tail	T cells; NK cells	Signaling chain of TCR- and NK cell–activating receptors
CD252 (OX40 ligand)	21 kD; TNF superfamily	DCs, macrophages, B cells	Ligand for CD134 (OX40, TNFRSF4); costimulates T cells
CD267 (TACI)	31 kD; TNFR superfamily	B cells	Receptor for cytokines BAFF and APRIL; mediates B cell survival
CD268 (BAFF receptor)	19 kD; TNFR superfamily	B cells	Receptor for BAFF; mediates B cell survival
CD269 (B cell maturation antigen [BCMA])	20 kD; TNFR superfamily	B cells	Receptor for BAFF and APRIL; mediates B cell survival
CD273 (PD-L2)	25 kD; Ig superfamily; structurally homologous to B7	DCs, monocytes, macrophages	Ligand for PD-1; inhibits T cell activation
CD274 (PD-L1)	33 kD; Ig superfamily; structurally homologous to B7	Leukocytes, other cells	Ligand for PD-1; inhibits T cell activation
CD275 (ICOS ligand)	60 kD; Ig superfamily; structurally homologous to B7	B cells, DCs, monocytes	Binds ICOS (CD278); T cell costimulation; Tfh cell development and function
CD278 (inducible costimulator [ICOS])	55–60 kD; Ig superfamily; structurally homologous to CD28	Activated T cells	Binds ICOS-L (CD275); T cell costimulation; Tfh cell development and function
CD279 (PD-1)	55 kD; Ig superfamily; structurally homologous to CD28	Activated T and B cells	Binds PD-L1 and PD-L2; inhibits T cell activation
CD303 (BDCA2,CLEC4C)	25 kD; C-type lectin superfamily	Plasmacytoid DCs	Binds to microbial carbohydrates; inhibits DC activation
CD304 (BDCA4, neuropilin)	103 kD; complement-binding, coagulation factor V/VIII, and meprin domains	Plasmacytoid DCs, many other cell types	Vascular endothelial growth factor A receptor
CD314 (NKG2D)	42 kD; C-type lectin	NK cells, activated CD8$^+$ T cells, NKT cells, some myeloid cells	Binds MHC class I, and the class I–like molecules MIC-A, MIC-B, Rae1, and ULBP4; role in NK cell and CTL activation
CD319 (SLAM-F7, CS1)	35 kD; SLAM family	Plasma cells, NK cells, activated CD8$^+$ T cells	Homophilic receptor; may play a role in activation of B and NK cells; target for immunotherapy of myeloma

Table Continued

CD Number (Other Names)	Molecular Structure, Family	Main Cellular Expression	Known or Proposed Function(s)
CD357 (GITR, TNFRSF18)	26 kD; TNFR superfamily	$CD4^+$ and $CD8^+$ T cells, Treg	Role in Treg function?
CD363 (type 1 sphingosine 1-phosphate receptor 1 [S1PR1])	42.8 kD; G protein–coupled, seven membrane-spanning receptor family	Lymphocytes, endothelial cells	Binds sphingosine 1-phosphate and mediates chemotaxis of lymphocytes out of lymphoid organs
CD365 (hepatitis A virus cellular receptor 1 [HAVCR1], TIM-1)	38.7 kD; Ig superfamily, T cell transmembrane, Ig, and mucin family, T cell transmembrane, immunoglobulin, and mucin family	T cells, kidney and testis	Receptor for several viruses; modulation of T cell responses
CD366 (hepatitis A virus cellular receptor 2 [HAVCR2], TIM-3)	33.4 kD; Ig superfamily, Ig superfamily, T cell transmembrane, Ig, and mucin family, T cell transmembrane, immunoglobulin, and mucin family	T cells, macrophages, DCs, NK cells	Receptor for several viruses; binds phosphatidylserine on apoptotic cells; inhibits of T cell responses
CD369 (CLEC7A, DECTIN 1)	27.6 kD; C-type lectin family	DCs, monocytes, macrophages, B cells	Pattern receptor specific for fungal and bacterial cell wall glucans

ADCC, Antibody-dependent cell-mediated cytotoxicity; *APRIL,* a proliferation-inducing ligand; *BAFF,* B cell–activating factor belonging to the TNF family; *CTL,* cytotoxic T lymphocyte; *DCs,* dendritic cells; *EGFR,* epidermal growth factor receptor; *FDCs,* follicular dendritic cells; *GITR,* glucocorticoid-induced TNFR-related; *gp,* glycoprotein; *GPI,* glycophosphatidylinositol; *HLA,* human leukocyte antigen; *HIV,* human immunodeficiency virus; *ICAM*, intercellular adhesion molecule; *Ig,* immunoglobulin; *IL,* interleukin; *iNK,* invariant natural killer; *ITAM,* immunoreceptor tyrosine-based activation motif; *ITIM,* immunoreceptor tyrosine-based inhibition motif; *LFA,* lymphocyte function-associated antigen; *LPS,* lipopolysaccharide; *MAdCAM,* mucosal addressin cell adhesion molecule; *MHC,* major histocompatibility complex; *NK* cells, natural killer cells; *NKT,* NK T cells; *PAMPs,* pathogen-associated molecular patterns; *PD-1,* programmed cell death protein-1; *PSGL-1,* P-selectin glycoprotein ligand 1; *SLAM,* signaling lymphocytic activation molecule; *TACI,* transmembrane activator and CAML interactor; *TCR,* T cell receptor; *Tfh,* T follicular helper; *TLR,* Toll-like receptor; *TNF,* tumor necrosis factor; *TNFR,* TNF receptor; *Treg,* regulatory T cell; *VCAM,* vascular cell adhesion molecule; *VLA,* very late activation.

The lowercase letters affixed to some CD numbers refer to CD molecules that are encoded by multiple genes or that belong to families of structurally related proteins.

Appendix II

Cytokines

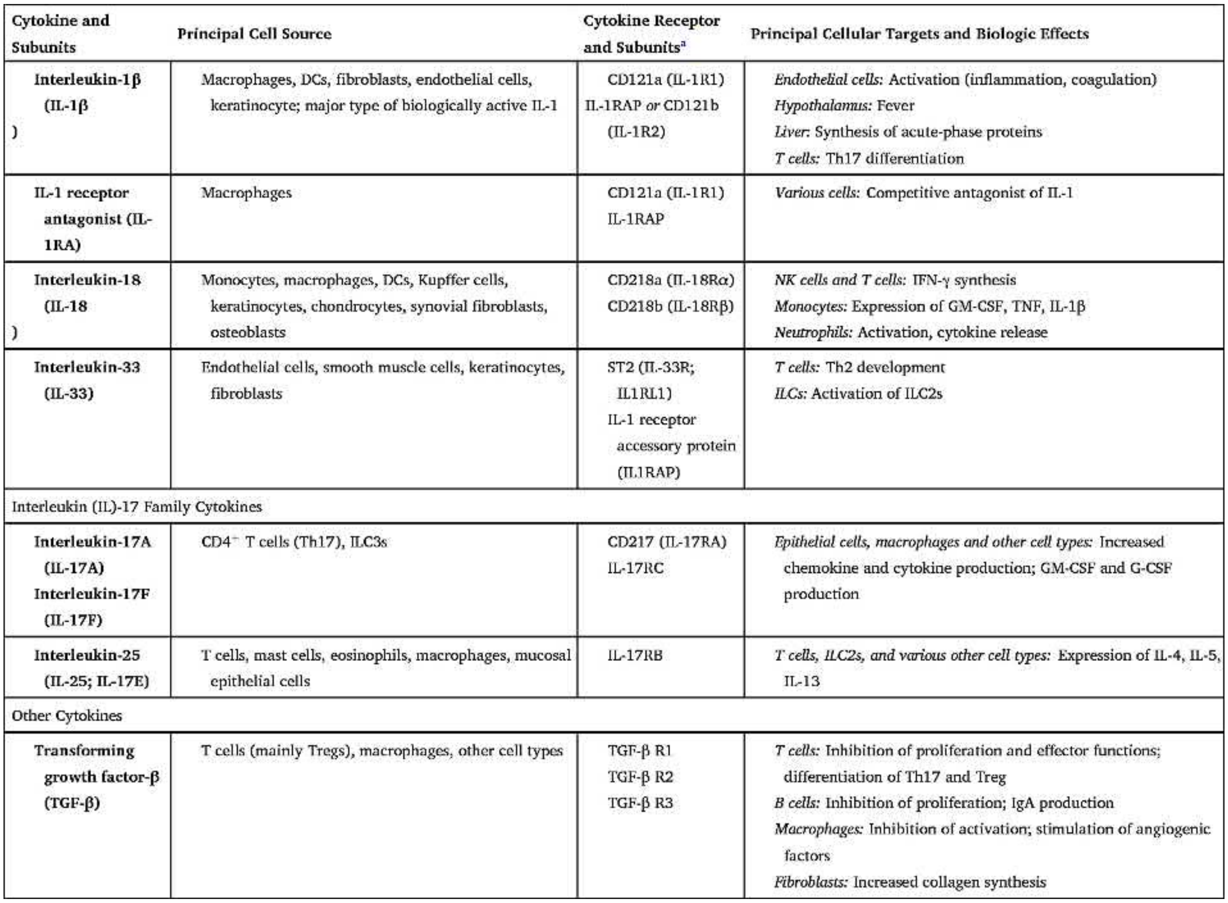
Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits[a]	Principal Cellular Targets and Biologic Effects
Type I Cytokine Family Members			
Interleukin-2 (IL-2)	T cells	CD25 (IL-2Rα) CD122 (IL-2Rβ) CD132 (γc)	*T cells:* Proliferation and differentiation into effector and memory cells; promotes regulatory T cell development, survival, and function *NK cells:* Proliferation, activation
Interleukin-3 (IL-3)	T cells	CD123 (IL-3Rα) CD131 (βc)	*Immature hematopoietic progenitors:* Induced maturation of all hematopoietic lineages
Interleukin-4 (IL-4)	CD4$^+$ T cells (Th2, Tfh), mast cells	CD124 (IL-4Rα) CD132 (γc)	*B cells:* Isotype switching to IgE, IgG4 (in humans; IgG1 in mice) *T cells:* Th2 differentiation, proliferation *Macrophages:* Alternative activation and inhibition of IFN-γ–mediated classical activation
Interleukin-5 (IL-5)	CD4$^+$ T cells (Th2), ILC2s	CD125 (IL-5Rα) CD131 (βc)	*Eosinophils:* Activation, increased generation
Interleukin-6 (IL-6)	Macrophages, DCs, endothelial cells, T cells	CD126 (IL-6Rα) CD130 (gp130)	*Liver:* Synthesis of acute-phase proteins *B cells:* Proliferation of antibody-producing cells *T cells:* Th17 differentiation
Interleukin-7 (IL-7)	Fibroblasts, bone marrow stromal cells	CD127 (IL-7R) CD132 (γc)	*Immature lymphoid progenitors:* Proliferation of early T and B cell progenitors *T lymphocytes:* Survival of naive and memory cells
Interleukin-9 (IL-9)	CD4$^+$ T cells	CD129 (IL-9R) CD132 (γc)	*Mast cells, B cells, T cells, and tissue cells:* Survival and activation
Interleukin-11 (IL-11)	Bone marrow stromal cells	IL-11Rα CD130 (gp130)	Production of platelets
Interleukin-12 (IL-12): IL-12A (p35)IL-12B (p40)	Macrophages, DCs	CD212 (IL-12Rβ1) IL-12Rβ2	*T cells:* Th1 differentiation *NK cells and T cells:* IFN-γ synthesis, increased cytotoxic activity

Table Continued

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits[a]	Principal Cellular Targets and Biologic Effects
Interleukin-13 (IL-13)	CD4$^+$ T cells (Th2), NKT cells, ILC2s, mast cells	CD213a1 (IL-13Rα1) CD213a2 (IL-13Rα2) CD132 (γc)	*B cells:* Isotype switching to IgE *Epithelial cells:* Increased mucus production *Macrophages:* Alternative activation
Interleukin-15 (IL-15)	Macrophages, other cell types	IL-15Rα CD122 (IL-2Rβ) CD132 (γc)	*NK cells:* Proliferation *T cells:* Survival and proliferation of memory CD8$^+$ cells
Interleukin-21 (IL-21)	Th2 cells, Th17 cells, Tfh cells	CD360 (IL-21R) CD132 (γc)	*B cells:* Activation, proliferation, and differentiation in the germinal center reaction
Interleukin-23 (IL-23): **IL-23A (p19)** **IL-12B (p40)**	Macrophages, DCs	IL-23R CD212 (IL-12Rβ1)	*T cells:* Differentiation and expansion of Th17 cells
Interleukin-27 (IL-27): **IL-27 (p28)** **EBI3 (IL-27B)**	Macrophages, DCs	IL-27Rα CD130 (gp130)	*T cells:* Enhancement of Th1 differentiation; inhibition of Th17 differentiation *NK cells:* Activation, IFN-γ synthesis
Stem cell factor (c-KIT ligand)	Bone marrow stromal cells	CD117 (KIT)	*Pluripotent hematopoietic stem cells:* Induced maturation of all hematopoietic lineages
Granulocyte CSF (G-CSF, CSF3)	Macrophages, fibroblasts, endothelial cells	CD114 (CSF3R)	*Committed hematopoietic progenitors:* Maturation of granulocytes
Granulocyte-monocyte CSF (GM-CSF)	T cells, macrophages, endothelial cells, fibroblasts	CD116 (GM-CSFRα) CD131 (βc)	*Immature and committed progenitors, mature macrophages:* Induced maturation of granulocytes and monocytes, macrophage activation
Monocyte CSF (M-CSF, CSF1)	Macrophages, endothelial cells, bone marrow cells, fibroblasts	CD115 (CSF1R)	*Committed hematopoietic progenitors:* Maturation of monocytes
Thymic stromal lymphopoietin (TSLP)	Keratinocytes, bronchial epithelial cells, fibroblasts, smooth muscle cells, endothelial cells, mast cells, macrophages, granulocytes, and DCs	TSLP-receptor CD127 (IL-7R)	*DCs:* Activation *Eosinophils:* Activation *Mast cells:* Cytokine production *T cells:* Th2 differentiation
Type II Cytokine Family Members			
IFN-α (type I IFN; multiple proteins)	Plasmacytoid DCs, macrophages	IFNAR1 CD118 (IFNAR2)	*All cells:* Antiviral state, increased class I MHC expression *NK cells:* Activation
IFN-β (type I IFN)	Fibroblasts, plasmacytoid DCs	IFNAR1 CD118 (IFNAR2)	*All cells:* Antiviral state, increased class I MHC expression *NK cells:* Activation

Table Continued

Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits[a]	Principal Cellular Targets and Biologic Effects
IFN-γ (type II IFN)	T cells (Th1, CD8$^+$ T cells), NK cells, ILC1s	CD119 (IFNGR1) IFNGR2	*Macrophages:* Classical activation (increased microbicidal functions) *B cells:* Isotype switching to opsonizing and complement-fixing IgG subclasses (in mice, not humans) *T cells:* Th1 differentiation *Various cells:* Increased expression of class I and class II MHC molecules, increased antigen processing and presentation to T cells
Interleukin-10 (IL-10)	Macrophages, T cells (mainly regulatory T cells)	CD210 (IL-10Rα) IL-10Rβ	*Macrophages, DCs:* Inhibition of expression of IL-12, costimulators, and class II MHC
Interleukin-22 (IL-22)	Th17 cells, ILC3s	IL-22Rα1 or IL-22Rα2 IL-10Rβ2	*Epithelial cells:* Production of defensins, increased barrier function *Hepatocytes:* Survival
Interferon-λs (type III IFNs)	DCs	IFNLR1 (IL-28Rα) CD210B (IL-10Rβ2)	*Epithelial cells:* Antiviral state
Leukemia inhibitory factor (LIF)	Embryonic trophoectoderm, bone marrow stromal cells	CD118 (LIFR) CD130 (gp130)	*Stem cells:* Block in differentiation
Oncostatin M	Bone marrow stromal cells	OSMR CD130 (gp130)	*Endothelial cells:* Upregulation of cytokine and adhesion molecule expression *Intestinal stromal cells:* production of inflammatory cytokines, chemokines *Cancer cells:* Inhibition of proliferation
Tumor Necrosis Factor (TNF) Superfamily Cytokines[b]			
APRIL (CD256, TNFSF13)	T cells, DCs, monocytes, follicular DCs	TACI (TNFRSF13B) or BCMA (TNFRSF17)	B cells: Survival, proliferation
BAFF (CD257, TNFSF13B)	DCs, monocytes, follicular DCs, B cells	BAFF-R (TNFRSF13C) or TACI (TNFRSF13B) or BCMA (TNFRSF17)	*B cells:* Survival, proliferation
Tumor necrosis factor (TNF, also called TNF-α; TNFSF2)	Macrophages, NK cells, T cells	CD120a (TNFR1, TNFRSF1) or CD120b (TNFR2, TNFRSF2)	*Endothelial cells:* Activation (inflammation, coagulation) *Neutrophils:* Activation *Hypothalamus:* Fever *Muscle, fat:* Catabolism (cachexia)
Lymphotoxin-α (LTα, TNFSF1)	T cells, B cells	CD120a (TNFR1, TNFRSF1) or CD120b (TNFR2, TNFRSF2)	Same as TNF
Lymphotoxin-αβ (LTαβ, LTα1β2,)	T cells, NK cells, follicular B cells, lymphoid inducer cells	LTβR	*Lymphoid tissue stromal cells and follicular DCs:* Chemokine expression and lymphoid organogenesis
Interleukin (IL)-1 Family Cytokines			
Interleukin-1α (IL-1α)	Macrophages, DCs, fibroblasts, endothelial cells, keratinocytes, hepatocytes	CD121a (IL-1R1) IL-1RAP *or* CD121b (IL-1R2)	*Endothelial cells:* Activation (inflammation, coagulation) *Hypothalamus:* Fever
			Table Continued



Cytokine and Subunits	Principal Cell Source	Cytokine Receptor and Subunits[a]	Principal Cellular Targets and Biologic Effects
Interleukin-1β (IL-1β)	Macrophages, DCs, fibroblasts, endothelial cells, keratinocyte; major type of biologically active IL-1	CD121a (IL-1R1) IL-1RAP *or* CD121b (IL-1R2)	*Endothelial cells:* Activation (inflammation, coagulation) *Hypothalamus:* Fever *Liver:* Synthesis of acute-phase proteins *T cells:* Th17 differentiation
IL-1 receptor antagonist (IL-1RA)	Macrophages	CD121a (IL-1R1) IL-1RAP	*Various cells:* Competitive antagonist of IL-1
Interleukin-18 (IL-18)	Monocytes, macrophages, DCs, Kupffer cells, keratinocytes, chondrocytes, synovial fibroblasts, osteoblasts	CD218a (IL-18Rα) CD218b (IL-18Rβ)	*NK cells and T cells:* IFN-γ synthesis *Monocytes:* Expression of GM-CSF, TNF, IL-1β *Neutrophils:* Activation, cytokine release
Interleukin-33 (IL-33)	Endothelial cells, smooth muscle cells, keratinocytes, fibroblasts	ST2 (IL-33R; IL1RL1) IL-1 receptor accessory protein (IL1RAP)	*T cells:* Th2 development *ILCs:* Activation of ILC2s
Interleukin (IL)-17 Family Cytokines			
Interleukin-17A (IL-17A) **Interleukin-17F (IL-17F)**	CD4$^-$ T cells (Th17), ILC3s	CD217 (IL-17RA) IL-17RC	*Epithelial cells, macrophages and other cell types:* Increased chemokine and cytokine production; GM-CSF and G-CSF production
Interleukin-25 (IL-25; IL-17E)	T cells, mast cells, eosinophils, macrophages, mucosal epithelial cells	IL-17RB	*T cells, ILC2s, and various other cell types:* Expression of IL-4, IL-5, IL-13
Other Cytokines			
Transforming growth factor-β (TGF-β)	T cells (mainly Tregs), macrophages, other cell types	TGF-β R1 TGF-β R2 TGF-β R3	*T cells:* Inhibition of proliferation and effector functions; differentiation of Th17 and Treg *B cells:* Inhibition of proliferation; IgA production *Macrophages:* Inhibition of activation; stimulation of angiogenic factors *Fibroblasts:* Increased collagen synthesis

APRIL, A proliferation-inducing ligand; *BAFF,* B cell–activating factor belonging to the TNF family; *BCMA,* B cell maturation protein; *CSF,* colony-stimulating factor; *DC,* dendritic cell; *IFN,* interferon; *IgE,* immunoglobulin E; *ILC,* innate lymphoid cell; *MHC,* major histocompatibility complex; *NK* cell, natural killer cell; *NKT* cell, natural killer T cell; *OSMR,* oncostatin M receptor; *RANK,* receptor activator for nuclear factor κB ligand; *RANKL,* RANK ligand; *TACI,* transmembrane activator and calcium modulator and cyclophilin ligand interactor; *Tfh,* T follicular helper cell; *Th,* helper T cell; *TNF,* tumor necrosis factor; *TNFSF,* TNF superfamily; *TNFRSF,* TNF receptor superfamily.

[a] Most cytokine receptors are dimers or trimers composed of different polypeptide chains, some of which are shared between receptors for different cytokines. The set of polypeptides that compose a functional receptor (cytokine binding plus signaling) for each cytokine is listed. The functions of each subunit polypeptide are not listed.

[b] All TNF superfamily (TNFSF) members are expressed as cell surface transmembrane proteins, but only those that are active as proteolytically released soluble cytokines are listed in the table. Other TNFSF members that function predominantly in the membrane-bound form and are not, strictly speaking, cytokines are not listed in the table. These membrane-bound proteins and the TNFRSF receptors they bind to include OX40L (CD252, TNFSF4):OX40 (CD134, TNFRSF4):CD40L (CD154, TNFSF5); CD40 (TNFRSF5); FASL (CD178, TNFSF6):FAS (CD95, TNFRSF6); CD70 (TNFSF7):CD27 (TNFRSF27); CD153 (TNFSF8):CD30 (TNFRSF8); TRAIL (CD253, TNFSF10):TRAIL-R (TNFRSF10A-D); RANKL (TNFSF11):RANK (TNFRSF11); TWEAK (CD257, TNFSF12):TWEAKR (CD266, TNFRSF12);

LIGHT (CD258, TNFSF14):HVEM (TNFRSF14); GITRL (TNFSF18):GITR (CD357TNFRSF18); 4-IBBL:4-IBB (CD137).

Appendix III

Laboratory Techniques Commonly Used in Immunology

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Immunologic techniques are used widely both in research laboratories and clinical settings, and many of these are based on the use of antibodies. In addition, many of the techniques of modern molecular biology have provided invaluable information about the immune system and are also now being used to analyze features of immunologic diseases for diagnostic purposes. We have mentioned these techniques often throughout the book. In this Appendix, we will describe the principles underlying some of the most commonly used laboratory methods in immunology. In addition, we will summarize how B and T lymphocyte responses are studied in the laboratory. Details of how to carry out various assays may be found in laboratory manuals and research papers.

Laboratory Methods Using Antibodies

The exquisite specificity of antibodies for particular antigens makes them valuable reagents for detecting, purifying, and quantitating antigens. Because antibodies can be produced against virtually any type of macromolecule and small chemical, antibody-based techniques may be used to study virtually any type of molecule in solution or in cells. The methods for producing monoclonal antibodies (see Chapter 5) have greatly increased our ability to generate antibodies of almost any desired specificity. Historically, many of the uses of antibody depended on the ability of antibody and specific antigen to form large immune complexes, either in solution or in gels, that could be detected by various optical methods. These methods were of great importance in early studies but have now been replaced almost entirely by simpler methods based on immobilized antibodies or antigens.

Quantitation of Antigen by Immunoassays

Immunologic methods of quantifying antigen concentration provide high sensitivity and specificity and have become standard techniques for both research and clinical applications. All modern immunochemical methods of quantitation are based on having a pure antigen or antibody whose quantity can be measured by an indicator molecule (or a label). When the antigen or antibody is labeled with a radioisotope, as first introduced by Rosalyn Yalow and colleagues, it may be quantified by instruments that detect radioactive decay events; the assay is called a **radioimmunoassay (RIA).** More commonly now, the antigen or antibody is covalently coupled to an enzyme and is quantified by determining with a spectrophotometer the rate at which the enzyme converts a clear substrate to a colored product; the assay is called an **enzyme-linked immunosorbent assay (ELISA).** Several variations of RIA and ELISA exist, but the most commonly used version is the sandwich assay (Fig. A.1). The sandwich assay uses two different antibodies reactive with different epitopes on the antigen whose concentration needs to be determined. A fixed quantity of one antibody is attached to a series of replicate solid supports, such as plastic microtiter wells. Test solutions containing antigen at an unknown concentration or a series of standard solutions with known concentrations of antigen are added to the wells and allowed to bind. Unbound antigen is removed by washing, and the second antibody, which is enzyme linked, radiolabeled, or photochemically labeled, is allowed to bind. The antigen serves as a bridge, so the more antigen in the test or standard solutions, the more enzyme-linked or radiolabeled second antibody will bind. The results from the standard solutions are used to construct a binding curve for the second antibody as a function of antigen concentration, from which the quantities of antigen in the test solutions may be inferred. When this test is performed with two monoclonal antibodies, it is essential that these antibodies see nonoverlapping determinants on the antigen; otherwise, the second antibody cannot bind. The sandwich ELISA approach has been adapted to many point-of-care and home tests, in which the enzyme generation of a colored product is readily determined by portable spectrophotometers. In most home tests, such as pregnancy tests, lateral flow technology is employed. The sample, such as a drop of urine, is added at one end of a matrix and diffuses linearly, first encountering pre-bound antibody specific for the analyte and labeled with a colored bead or chemical. Then complexes of the labeled antibody and bound analyte diffuse further until they are immobilized by a second analyte-specific antibody, forming a colored line indicating a positive test. A variation of this technology is a competitive test in which the analyte in the sample competes with pre-added analyte, and the absence of a colored line indicates a positive result.

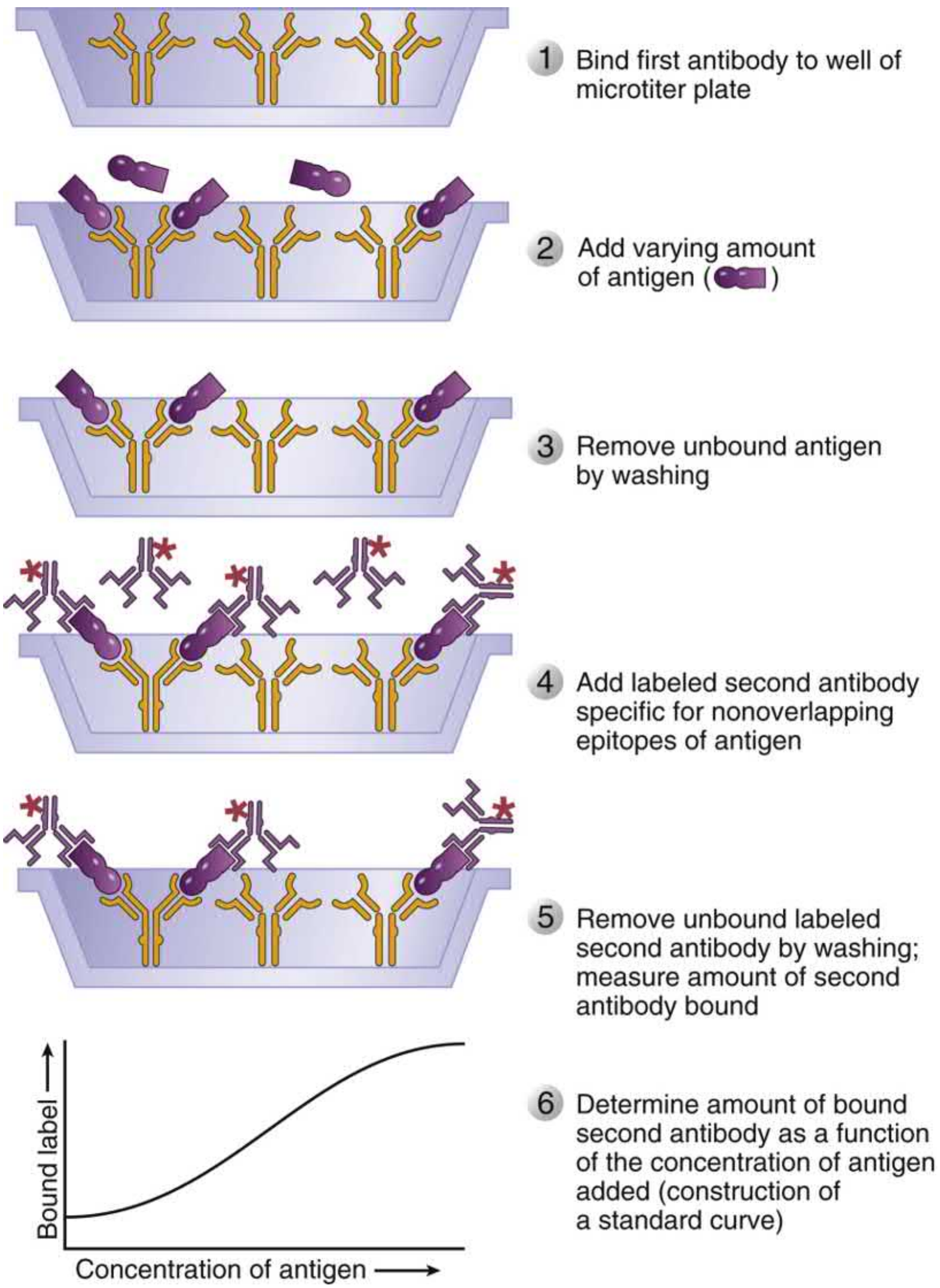

1 Bind first antibody to well of microtiter plate
2 Add varying amount of antigen ( )
3 Remove unbound antigen by washing
4 Add labeled second antibody specific for nonoverlapping epitopes of antigen
5 Remove unbound labeled second antibody by washing; measure amount of second antibody bound
6 Determine amount of bound second antibody as a function of the concentration of antigen added (construction of a standard curve)
Bound label
Concentration of antigen

FIG. A1 Sandwich enzyme-linked immunosorbent assay or radioimmunoassay.A fixed amount of one immobilized antibody is used to capture an antigen. The binding of a second, labeled antibody that recognizes a nonoverlapping determinant on

the antigen will increase as the concentration of antigen increases and thus allow quantification of the antigen.

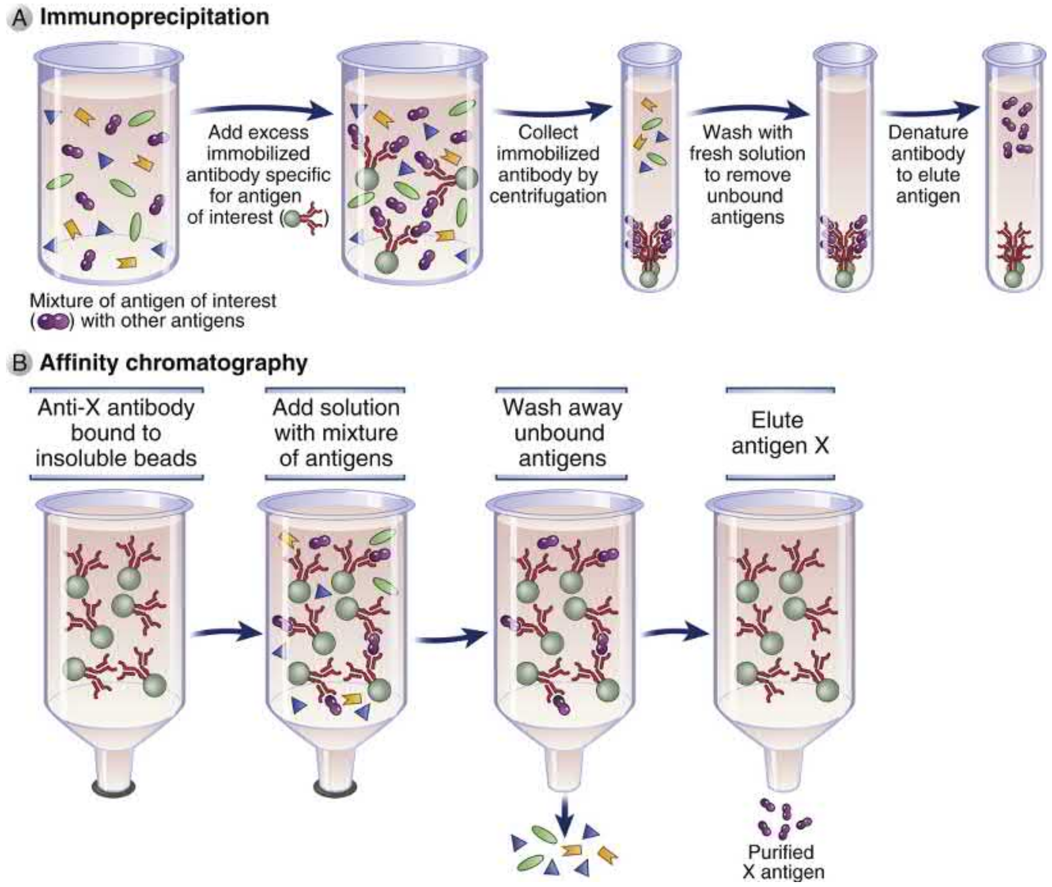

A Immunoprecipitation
Add excess immobilized antibody specific for antigen of interest
Collect immobilized antibody by centrifugation
Wash with fresh solution to remove unbound antigens
Denature antibody to elute antigen
Mixture of antigen of interest ( ) with other antigens
B Affinity chromatography
Anti-X antibody bound to insoluble beads
Add solution with mixture of antigens
Wash away unbound antigens
Elute antigen X
Purified X antigen

FIG. A.2 Isolation of an antigen by immunoprecipitation or affinity chromatography. **A,** A particular antigen can be purified from a mixture of antigens in serum or other solutions by adding antibodies specific to the antigen that are bound to insoluble beads. Unbound antigens are then washed away, and the desired antigen is recovered by changing the pH or ionic strength of the solution so that the affinity of antibody-antigen binding is lowered. Immunoprecipitation can be used as a means of purification, as a means of quantification, or as a means of identification of an antigen. Antigens purified by immunoprecipitation are often analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. **B,** Affinity chromatography is based on the same principle as immunoprecipitation, except that the antibody

is fixed to an insoluble matrix or beads, usually in a column. The method is often used to isolate soluble antigens *(shown)* or antibodies specific for an immobilized antigen.

In an important clinical variant of immunobinding assays, samples from patients may be tested for the presence of antibodies that are specific for a microbial antigen (e.g., antibodies reactive with proteins from human immunodeficiency virus [HIV] or hepatitis B virus) as indicators of infection. In this case, a saturating quantity of antigen is added to replicate wells containing plate-bound antibody, or the antigen is attached directly to the plate, and serial dilutions of the patient's serum are then allowed to bind. The amount of the patient's antibody bound to the immobilized antigen is determined by use of an enzyme-linked anti-human immunoglobulin (Ig) antibody.

Identification and Purification of Proteins

Antibodies can be used to identify and characterize proteins and to purify specific proteins from mixtures. Two commonly used methods to identify and purify proteins are immunoprecipitation and immuno-affinity chromatography. Western blotting is a widely used technique to determine the presence and size of a protein in a biologic sample.

Immunoprecipitation and Immuno-Affinity Chromatography

Immunoprecipitation is a technique in which an antibody specific for one protein antigen in a mixture of proteins is used to identify this specific antigen (Fig. A.2A). The antibody is typically added to a protein mixture (usually a detergent lysate of specific cells), and staphylococcal protein A or protein G covalently attached to agarose beads is added to the mixture. The Fab portions of the antibody bind to the target protein, and the Fc portion of the antibody is captured by the protein A or protein G on the beads. Unwanted proteins that do not bind to the antibody are then removed by washing the beads (by repeated detergent addition and centrifugation). Alternatively, superparamagnetic beads coated with protein A or G can be used instead of agarose beads, and after being added to a protein mixture, the beads can be isolated by powerful magnets. The specific protein that is recognized by and bound to the antibody may be eluted from the agarose or magnetic beads and dissociated from the antibody by use of a harsh denaturant (such as sodium dodecyl sulfate), and the proteins are separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Proteins may be detected after electrophoresis by staining the polyacrylamide gel with a protein stain or by Western blot analysis (described later). If the original mixture contained radioactively labeled proteins, specific proteins immunoprecipitated by the antibody may be revealed by autofluorography or autoradiography, with protein bands being captured on x-ray film placed on the dried SDS–polyacrylamide gel containing separated proteins.

Immuno-affinity chromatography, a variant of affinity chromatography, is a purification method that relies on antibodies attached to an insoluble support to purify antigens from a solution (Fig. A.2B). Antibodies specific for the desired antigen are typically covalently attached to a solid support, such as agarose beads, and packed into a column. A complex mixture of antigens is passed through the beads to allow the antigen that is recognized by the antibody to bind. Unbound molecules are washed away, and the bound antigen is eluted by changing the pH or by exposure to high salt or other chaotropic conditions that break antigen-antibody interactions. A similar method may be used to purify antibodies from culture supernatants or natural fluids, such as serum, by first attaching the antigen to beads and passing the supernatants or serum through. The identity of the purified protein can be conclusively determined by using mass spectrometry. This approach has been used to identify specific antigens with certainty.

In addition, immunoprecipitation combined with mass spectrometry can accurately identify all the proteins that are in a complex with the protein being identified by a specific antibody.

Western Blotting

Western blotting (Fig. A.3) is used to identify and determine the relative quantity and molecular weight of a protein within a mixture of proteins or other molecules. The mixture is first subjected to analytical separation, typically by SDS-PAGE, so that the final positions of different proteins in the gel are a function of their molecular size. The array of separated proteins is then transferred from the separating polyacrylamide gel to a support membrane by electrophoresis such that the membrane acquires a replica of the array of separated macromolecules present in the gel. SDS is displaced from the protein during the transfer process, and native antigenic determinants are often regained as the protein refolds. The position of the protein antigen on the membrane can then be detected by binding of an unlabeled antibody specific for that protein (the primary antibody) followed by a labeled second antibody that binds to the primary antibody. This approach provides information about antigen size and relative quantity (which, with use of quantitative scanning, can be accurate and reproducible). In general, second antibody probes are labeled with enzymes that generate chemiluminescent signals and leave images on photographic film. Near-infrared fluorophores also can be used to label antibodies, and light produced by the excitation of the fluorophore provides more accurate antigen quantitation compared with enzyme-linked second antibodies. The sensitivity and specificity of this technique can be increased by starting with immunoprecipitated proteins instead of crude protein mixtures. This sequential procedure is especially useful for detecting protein-protein interactions. For example, the physical association of two different proteins in the membrane of a lymphocyte can be established by immunoprecipitating a membrane extract by use of an antibody specific for one of the proteins and probing a Western blot of the immunoprecipitate using an antibody specific for the second protein that may have been co-immunoprecipitated along with the first protein.

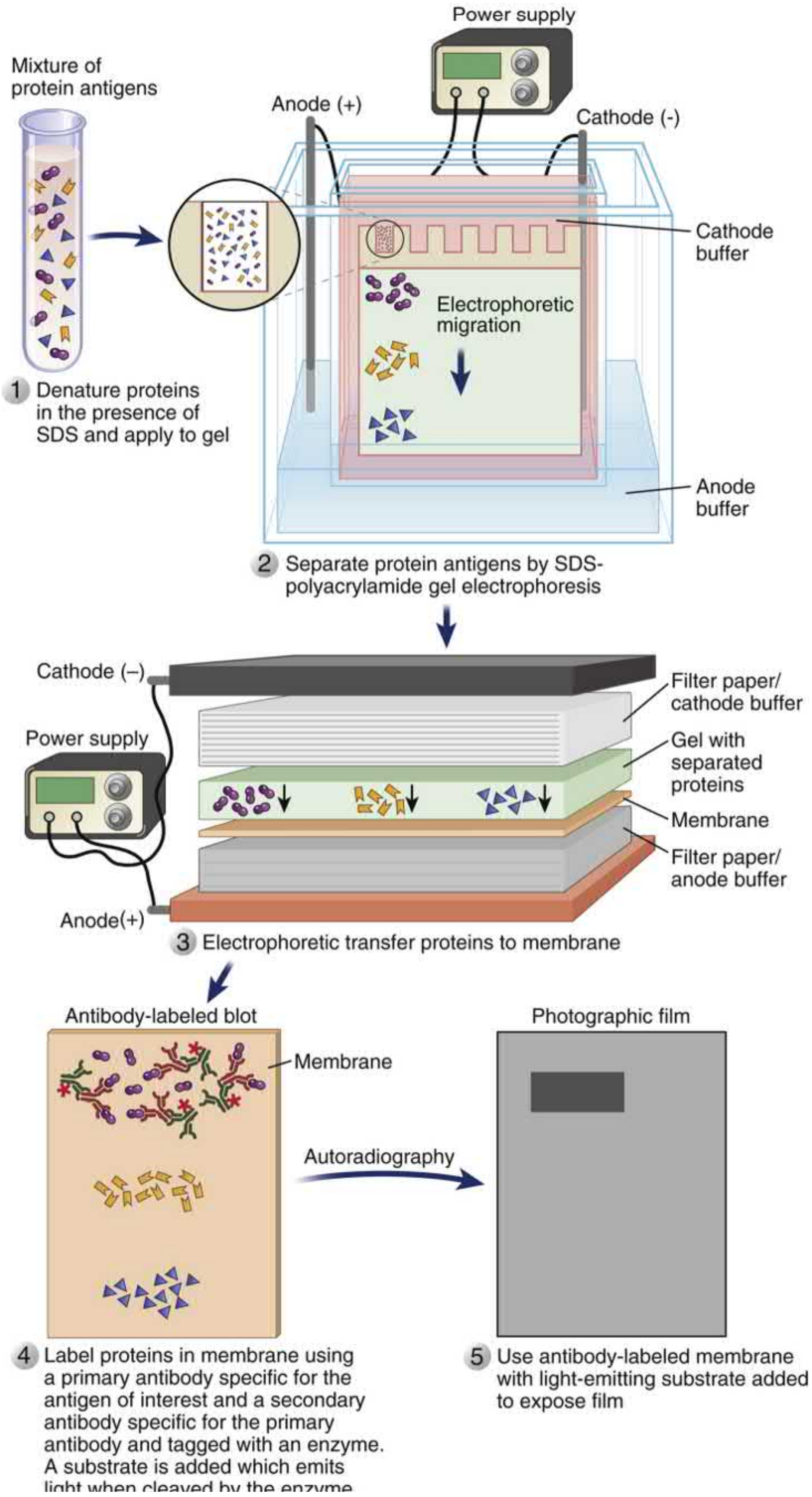
The technique of transferring proteins from a gel to a membrane is called Western blotting as a biochemist's joke. Southern is the last name of the scientist who first blotted DNA from a separating gel to a membrane by capillary transfer, a technique since called Southern blotting. By analogy, Northern blotting was the term applied to the technique of transferring RNA from a gel to a membrane, and Western blotting is the term used to describe the transfer of proteins to a membrane.

Labeling and Detection of Antigens in Cells and Tissues

Antibodies specific for antigens expressed on or in particular cell types are commonly used to identify these cells in tissues or cell suspensions and to separate these cells from mixed populations. In these methods, the antibody can be radiolabeled, enzyme linked, or, most commonly, fluorescently labeled, and a detection system is used that can identify the bound antibody. Antibodies attached to magnetic beads can be used to physically isolate cells expressing specific antigens.

Flow Cytometry

The lineage, maturation stage, or activation status of a cell often can be determined by analyzing the expression of different cell surface or intracellular molecules. This is commonly done by staining cells with fluorescently labeled probes that are specific for those molecules and measuring the quantity of fluorescence emitted by the cells (Fig. A.4). The flow cytometer is a specialized instrument that can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule to which a fluorescent probe binds as well as the level of expression of the molecule in each cell. Suspensions of cells are incubated with fluorescently labeled probes, and the amount of probe bound by each cell in the population is measured by passing the cells one at a time through a fluorimeter with a laser-generated incident beam. The relative amounts of a particular molecule on different cell populations can be compared by staining each population with the same probe and determining the amount of fluorescence emitted. In preparation for flow cytometric analysis, cell suspensions are stained with the fluorescent probes of choice. Most often, these probes are fluorochrome-labeled antibodies specific for a cell surface molecule. Alternatively, cytoplasmic molecules can be stained by temporarily permeabilizing cells and permitting the labeled antibodies to enter through the plasma membrane. In addition to antibodies, various fluorescent indicators of cytoplasmic ion concentrations and reduction-oxidation potential can be detected by flow cytometry. Cell cycle studies can be performed by flow cytometric analysis of cells stained with fluorescent DNA-binding probes, such as propidium iodide. Apoptotic cells can be identified with fluorescent probes, such as annexin V, that bind to abnormally exposed phospholipids on the surface of the dying cells. Modern flow cytometers routinely detect up to 30 different-colored fluorescent labels, each attached to a different antibody or other probe. This technique permits simultaneous analysis of the expression of many different combinations of molecules by a cell. In addition to detecting fluorescent signals, flow cytometers also measure the forward and side light-scattering properties of cells, which reflect cell size and internal complexity, respectively. This information is often used to distinguish different cell types. For example, compared with lymphocytes, neutrophils cause greater side scatter because of their cytoplasmic granules, and monocytes cause greater forward scatter because of their size. The light scatter and fluorescence data generated by the flow cytometer are analyzed by dedicated software to define subsets of cells expressing different combinations of molecules and to quantify the relative amounts of each molecule present in or on each cell.


Mixture of protein antigens
Power supply
Anode (+)
Cathode (-)
Cathode buffer
Electrophoretic migration
Anode buffer
1 Denature proteins in the presence of SDS and apply to gel
2 Separate protein antigens by SDS-polyacrylamide gel electrophoresis
Cathode (–)
Power supply
Filter paper/ cathode buffer
Gel with separated proteins
Membrane
Filter paper/ anode buffer
Anode(+)
3 Electrophoretic transfer proteins to membrane
Antibody-labeled blot
Membrane
Autoradiography
Photographic film
4 Label proteins in membrane using a primary antibody specific for the antigen of interest and a secondary antibody specific for the primary antibody and tagged with an enzyme. A substrate is added which emits light when cleaved by the enzyme
5 Use antibody-labeled membrane with light-emitting substrate added to expose film

light when cleaved by the enzyme.

FIG. A.3 Characterization of antigens by Western blotting. Protein antigens, separated by sodium dodecyl sulfate *(SDS)*–polyacrylamide gel electrophoresis and transferred to a membrane, can be detected by an antibody that is in turn revealed by a second antibody that may be conjugated to an enzyme such as horseradish peroxidase or to a fluorophore.

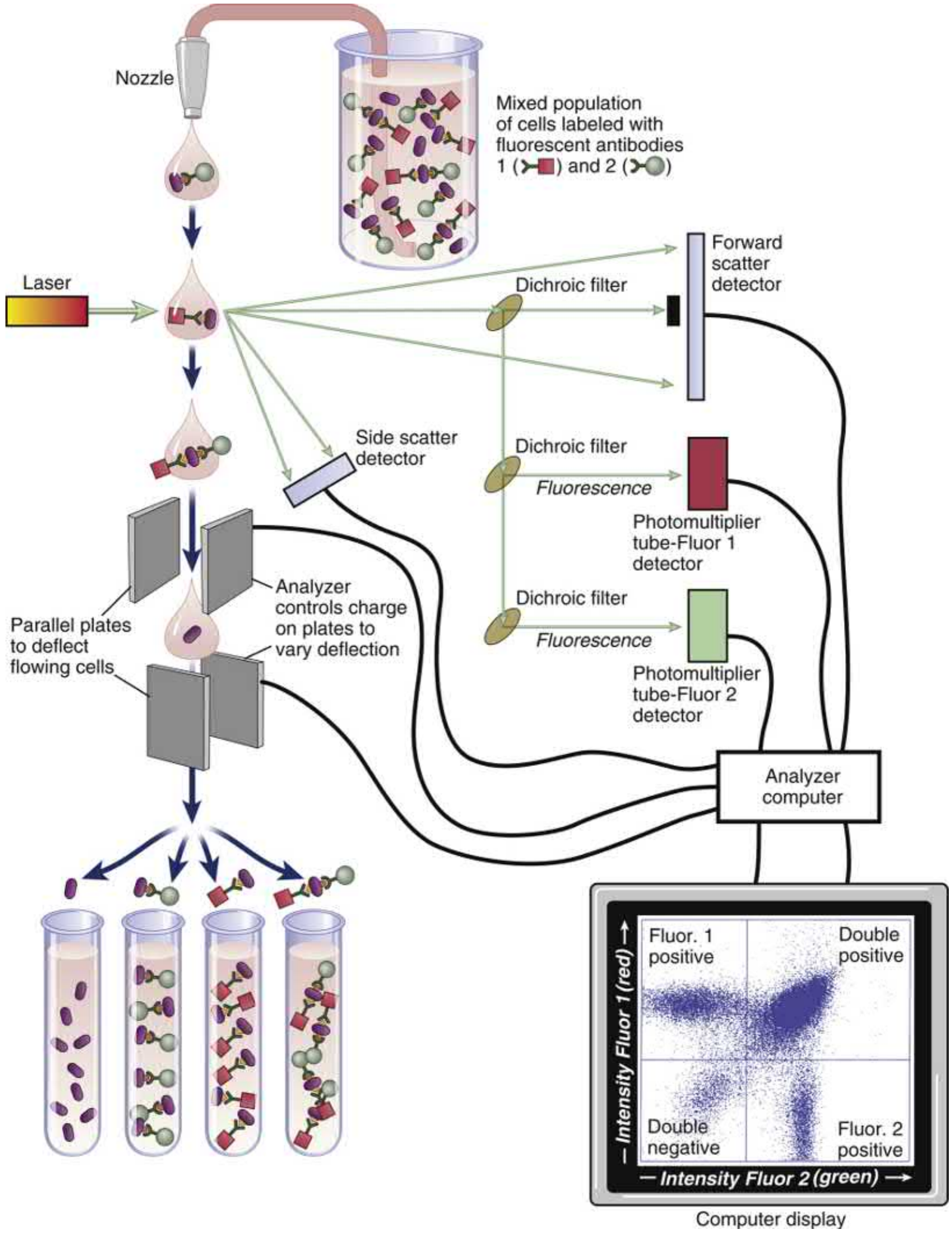

Nozzle
Mixed population of cells labeled with fluorescent antibodies 1 (>-■) and 2 (>-●)
Laser
Forward scatter detector
Dichroic filter
Side scatter detector
Dichroic filter
Fluorescence
Photomultiplier tube-Fluor 1 detector
Dichroic filter
Fluorescence
Photomultiplier tube-Fluor 2 detector
Parallel plates to deflect flowing cells
Analyzer controls charge on plates to vary deflection
Analyzer computer
Fluor. 1 positive
Double positive
Double negative
Fluor. 2 positive
— Intensity Fluor 1 (red) →
— Intensity Fluor 2 (green) →
Computer display

FIG. A.4 Principle of flow cytometry and fluorescence-activated cell sorting. The incident laser beam is of a designated wavelength, and the light that emerges from the sample is analyzed for forward and side scatter as well as fluorescent light of two or more wavelengths that depend on the fluorochrome labels attached to the

antibodies. The separation depicted here is based on two antigenic markers (two-color sorting). Modern instruments can routinely analyze and separate cell populations on the basis of three or more different-colored probes.

A newly developed antibody-based technology called mass cytometry combines the single-cell flow technology of flow cytometers with mass spectrometry. The commercially available device used for this purpose is called **CyTOF,** with “TOF” indicating that it is a time-of-flight type of mass cytometer. Antibodies specific for molecules of interest are labeled with any one of a large number of heavy metals, using a different isotope for each antibody specificity. These antibodies are incubated with the cell population being studied, and the cells are analyzed by a CyTOF instrument that performs mass spectrometry on individual cells. Unlike fluorescence labels, many different heavy metal isotopes can be resolved by mass spectrometry without overlap, allowing for the simultaneous detection of as many as 100 different molecules in a single cell suspension. The data generated by CyTOF instruments requires bioinformatic software analysis to define and quantify subsets of the analyzed cells expressing different combinations of the molecules detected by the labeled antibodies.

Cytokine Bead Assays

In these assays, the concentration of many different cytokines (and other proteins) in a single small volume of solution can be determined simultaneously. Microscopic-sized beads of different sizes are labeled with different amounts of a fluorochrome, such as allophycocyanin (APC), and the beads are pre-coated with a cytokine-specific antibody. The beads with each anti-cytokine specificity can be distinguished from one another based on size and fluorescence intensity. These beads are mixed with the test solution that contains multiple cytokines, such as serum or supernatants of lymphocyte cultures. Each cytokine will bind only to beads of one particular size and fluorescence intensity. Biotinylated detection antibodies specific for each cytokine are added to form antibody-antigen sandwiches, and phycoerythrin (PE)-conjugated streptavidin is added to detect these sandwiches. The beads are simultaneously analyzed by a two-laser flow-based detection instrument. One laser identifies the bead and determines the cytokine being detected. The other laser measures the intensity of the PE-fluorescence signal, which is directly related to the amount of cytokine bound. Standard solutions with known concentrations of the cytokines are used to develop standard curves from which the quantity of cytokine in the test sample can be determined.

Purification of Cells

A **fluorescent-activated cell sorter (FACS)** is an adaptation of the flow cytometer that allows one to separate cell populations according to which and how much fluorescent probe they bind. This technique is accomplished by differentially deflecting the cells with electromagnetic fields whose strength and direction are varied according to the measured intensity of the fluorescent signal (see Fig. A.4). The cells may be labeled with fluorescently tagged antibodies ex vivo, or, in the case of experimental animal studies, labeling may be accomplished in vivo by expression of transgenes that encode fluorescent proteins, such as green fluorescent protein. (Transgenic technology is described later in this Appendix.)

Another commonly used technique to purify cells with a particular phenotype relies on antibodies that are attached to magnetic beads. These “immunomagnetic reagents” will bind to certain cells, depending on the specificity of the antibody used, and the bound cells can then be pulled out of suspension by a strong magnet.

Immunofluorescence and Immunohistochemistry

Antibodies can be used to identify the anatomic distribution of an antigen within a tissue or within compartments of a cell. To do so, the tissue or cell is incubated with an antibody that is labeled with a fluorochrome or enzyme, and the position of the label, determined with a suitable microscope, is used to infer the position of the antigen. In the earliest version of this method, called immunofluorescence, the antibody was labeled with a fluorescent dye and allowed to bind to a monolayer of cells or to a frozen section of a tissue. The stained cells or tissues were examined with a fluorescence microscope to locate the antibody. Sensitivity has also increased dramatically with the use of enzymatic amplification methods for the detection of fluorescent labels. Immunofluorescence microscopes are now also used in combination with automated slide scanning devices and multispectral imaging devices that can deconvolute signals from fluorophores of very similar wavelengths, allowing a very large number of fluorescent probes to be used in the multicolor staining of tissues. It is now possible to visualize and quantitate for instance, every known T cell subset as well as different B cells and macrophages in a single slide form a fixed tissue using antigen restoration approaches, multicolor immunofluorescence with signal amplification, slide scanning devices, and multispectral imagers. Although sensitive, the fluorescence microscope not an ideal tool to identify the detailed structures of the cell or tissue because of a low signal-to-noise ratio. More granular analysis of intracellular structures can be ascertained by confocal microscopy, which uses optical sectioning technology to filter out unfocused fluorescent light, and live imaging of tissues is possible using multiphoton microscopy, which prevents out-of-focus light from forming.

Another classical approach to the analysis of antigens in tissues involves the use of antibodies coupled to enzymes that convert colorless substrates to colored insoluble substances that precipitate at the position of the enzyme, but this approach to immunohistochemical analyses allows limited number of protein antigens to be visualized at one time. A conventional light microscope may then be used to localize the antibody in a stained cell or tissue. The most common variant of this method uses the enzyme horseradish peroxidase, and the method is commonly referred to as the immunoperoxidase technique. Another commonly used enzyme is alkaline phosphatase. Different antibodies coupled to different enzymes may be used in conjunction to produce simultaneous two-color localizations of different antigens. In other variations, antibody can be coupled to an electron-dense probe such as colloidal gold, and the location of antibody can be determined subcellularly by means of an electron microscope, a technique called immunoelectron microscopy. Different-sized gold particles have been used for simultaneous localization of different antigens at the ultrastructural level.

In all immunomicroscopic methods, signals may be enhanced by use of sandwich techniques. For example, instead of attaching horseradish peroxidase to a specific mouse antibody directed against the antigen of interest, it can be attached to a second anti-antibody (e.g., rabbit anti-mouse Ig antibody) that is used to bind to the first, unlabeled antibody. When the label is attached directly to the specific, primary antibody, the method is referred to as direct; when the label is attached to a secondary or even tertiary antibody, the method is indirect. In some cases, molecules other than antibody can be used in indirect methods. For example, staphylococcal protein A, which binds to IgG, or avidin, which binds to primary antibodies labeled with biotin, can be coupled to fluorochromes or enzymes.

A new version of these methods uses isotope-labeled antibodies and is called multiplexed ion beam imaging (MIBI). This method can simultaneously detect up to 40 antigens and thus define the spatial distribution of many different cell types and molecules in a tissue.

Measurement of Antigen-Antibody Interactions

In many situations, it is important to know the affinity of an antibody for an antigen, that is, the strength of binding of a single antibody to an antigen. Affinity is measured by and expressed as the equilibrium dissociation constant (K_d). The lower the K_d, the higher the affinity. The effectiveness of antibodies in protecting against infection, or usefulness of a monoclonal antibody as an experimental or therapeutic reagent, depend on affinity. Antibody affinities for small antigens (e.g., haptens) were previously measured by a method called equilibrium dialysis involving two fluid-filled compartments separated by a semi-permeable membrane that allows passage of the antigen but not antibody. A known quantity of antigen is placed in one compartment, and of a known antibody is placed in the other compartment. After some time, equilibrium is achieved, and the concentration of the unbound antigen is equal in both compartments, but there is an additional amount of antigen in the antibody compartment, which cannot diffuse through the membrane out of the compartment because it is bound to the antibody. The amount of this additional antigen depends on the original free antigen concentration, the antibody concentration, and on the dissociation constant (K_d) of the binding interaction. The K_d can be calculated by measurement of antigen and antibody concentrations, using spectroscopy, or by other means involving labels on the antibody and antigen.

A newer and now more commonly used way to determine K_d is by measurement of the rates of antigen-antibody complex formation and dissociation on a solid surface using special optical techniques, such as surface plasma resonance (SPR) or polarization-modulated oblique-incidence reflectivity difference (OI-DR). In these techniques, antigen or antibody is immobilized on a solid thin metal surface (in SPR) or glass surface (in OI-DR) and ligand (antibody or antigen) is passed over the surface. A focused monochromatic laser beam is shone on the surface at a specific angle and the reflected light is analyzed. The changes in the surface that occur due to antigen-antibody complex formation and dissociation cause characteristic changes (distinct for each method) in the reflected light. These changes are used to determine rates of immune complex formation and dissociation, which depend, in part, on the concentrations of antibody and antigen and on the affinity of the interaction. The on-rate constant (K_{on}) and the off-rate constant (K_{off}) can be calculated by determining the actual rates of association or dissociation, respectively using the optical techniques. The ratio of $K_{\text{off}}/K_{\text{on}}$ is equal to the dissociation constant K_{d}.

Transgenic Mice and Gene Targeting

Three important and related methods for studying the functions of specific gene products in vivo are the creation of conventional transgenic mice that ectopically express a particular gene in a defined tissue; the creation of gene “knockout” mice, in which a targeted disruption is used to ablate the function of a particular gene; and the generation of “knockin” mice, in which an existing gene in the germline is replaced with a modified version of the same gene. A knockin approach could either replace a normal version of a gene with a mutant version or, in principle, “correct” an existing mutant gene with a “normal” version. These techniques involving genetically engineered mice have been widely used to analyze many biologic phenomena, including many aspects innate and adaptive immune responses.

For the creation of conventional **transgenic mice,** foreign DNA sequences, called transgenes, are introduced into the pronuclei of fertilized mouse eggs, and the eggs are implanted into the oviducts of pseudopregnant females. Usually, if a few hundred copies of a gene are injected into pro-nuclei, about 25% of the mice that are born are transgenic. One to 50 copies of the transgene insert in tandem into a random site of breakage in a chromosome and are subsequently inherited as a simple Mendelian trait. Because integration usually occurs before DNA replication, most (~75%) of the transgenic pups carry the transgene in all of their cells, including germ cells. In most cases, integration of the foreign DNA does not disrupt endogenous gene function. Also, each founder mouse carrying the transgene is a heterozygote, from which homozygous lines can be bred.

The great value of transgenic technology is that it can be used to express genes in particular tissues by attaching coding sequences of the gene to regulatory sequences that normally drive the expression of genes selectively in that tissue. For instance, lymphoid promoters and enhancers can be used to overexpress genes, such as rearranged antigen receptor genes, in lymphocytes, and the insulin promoter can be used to express genes in the β cells of pancreatic islets. Transgenes also can be expressed under the control of promoter elements that respond to drugs or hormones, such as tetracycline or estrogens. In these cases, transcription of the transgene can be controlled at will by administration of the inducing agent. A disadvantage of some transgenic approaches is that often the transgene product is not expressed with the same kinetics or at the same level as the endogenous protein, so experimental results may be difficult to interpret.

A powerful method for developing animal models of single-gene disorders, and the definitive way to establish the obligatory function of a gene product in vivo, is the creation of **knockout mice** by targeted mutation or disruption of the gene. This technique has mostly relied on the phenomenon of homologous recombination. If an exogenous gene is inserted into a cell, for instance, by electroporation, it can integrate randomly into the cell's genome. However, if the gene contains sequences that are homologous to an endogenous gene, it will preferentially recombine with and replace endogenous sequences. To select for cells that have undergone homologous recombination, a drug-based selection strategy is used. The fragment of homologous DNA to be inserted into a cell is placed in a vector typically containing a neomycin resistance *(neo)* gene and a viral thymidine kinase *(tk)* gene (Fig. A.5A). This targeting vector is constructed in such a way that the *neo* gene is always inserted into the chromosomal DNA, but the *tk* gene is lost whenever homologous recombination (as opposed to random insertion) occurs. The vector is introduced into cells, and the cells are grown in neomycin and ganciclovir, a drug that is metabolized by thymidine kinase to generate a lethal product. Cells in which the gene is integrated randomly will be resistant to neomycin but will be killed by ganciclovir, whereas cells in which homologous recombination has occurred will be resistant to both drugs because the *neo* gene will be expressed but the *tk* gene will not. This positive-negative selection ensures that the inserted gene in surviving cells has undergone homologous recombination with endogenous sequences. The presence of the inserted DNA in the middle of an endogenous gene usually disrupts the coding sequences and ablates the expression or function of that gene. In addition, targeting vectors can be designed such that homologous recombination will lead to the deletion of one or more exons of the endogenous gene.

To generate a mouse carrying a targeted gene disruption or mutation, a targeting vector is used to first disrupt the gene in a murine embryonic stem (ES) cell line. ES cells are pluripotent cells derived from mouse embryos that can be propagated and induced to differentiate in culture or that can be incorporated into a mouse blastocyst, which may be implanted in a pseudopregnant mother and carried to term. Importantly, the progeny of the ES cells develop normally into mature tissues that will express the exogenous genes that have been transfected into the ES cells. Thus, the targeting vector designed to disrupt a particular gene is inserted into ES cells, and colonies in which homologous recombination has occurred (on one chromosome) are selected with drugs, as described earlier (Fig. A.5B). The presence of the desired recombination is verified by analysis of DNA with techniques such as Southern blot hybridization or polymerase chain reaction. The selected ES cells are injected into blastocysts, which are implanted into pseudopregnant females. Mice that develop will be chimeric for a heterozygous disruption or mutation; that is, some of the tissues will be derived from the ES cells and others from the remainder of the normal blastocyst. The germ cells are also usually chimeric, but because these cells are haploid, only some will contain the chromosome copy with the disrupted (mutated) gene. If chimeric mice are mated with normal (wild-type) animals and either sperm or eggs containing the chromosome with the mutation fuse with the wild-type partner, all cells in the offspring derived from such a zygote will be heterozygous for the mutation (so-called germline transmission). Such heterozygous mice can be mated to yield animals that will be homozygous for the mutation with a frequency that is predictable by simple Mendelian segregation. Such knockout mice are deficient in expression of the targeted gene.

Homologous recombination also can be used to replace a normal gene sequence with a modified version of the same gene (or of another gene), thereby creating a knockin mouse strain. Knockin mice can be used to assess the biologic consequences of a change in a single base, for instance, as opposed to the deletion of a gene. A knockin approach also, in principle, could be used to replace a defective gene with a normal one. In certain circumstances, a different gene may be placed at a defined site in the genome by use of a knockin strategy rather than in a random site as in conventional transgenic mice. Knockin approaches are used when it is desirable to have the expression of the transgene regulated by certain endogenous DNA sequences, such as a particular enhancer or promoter region. In this case, the targeting vector contains an exogenous gene encoding a desired product as well as sequences homologous to an endogenous gene that are needed to target the site of recombination.

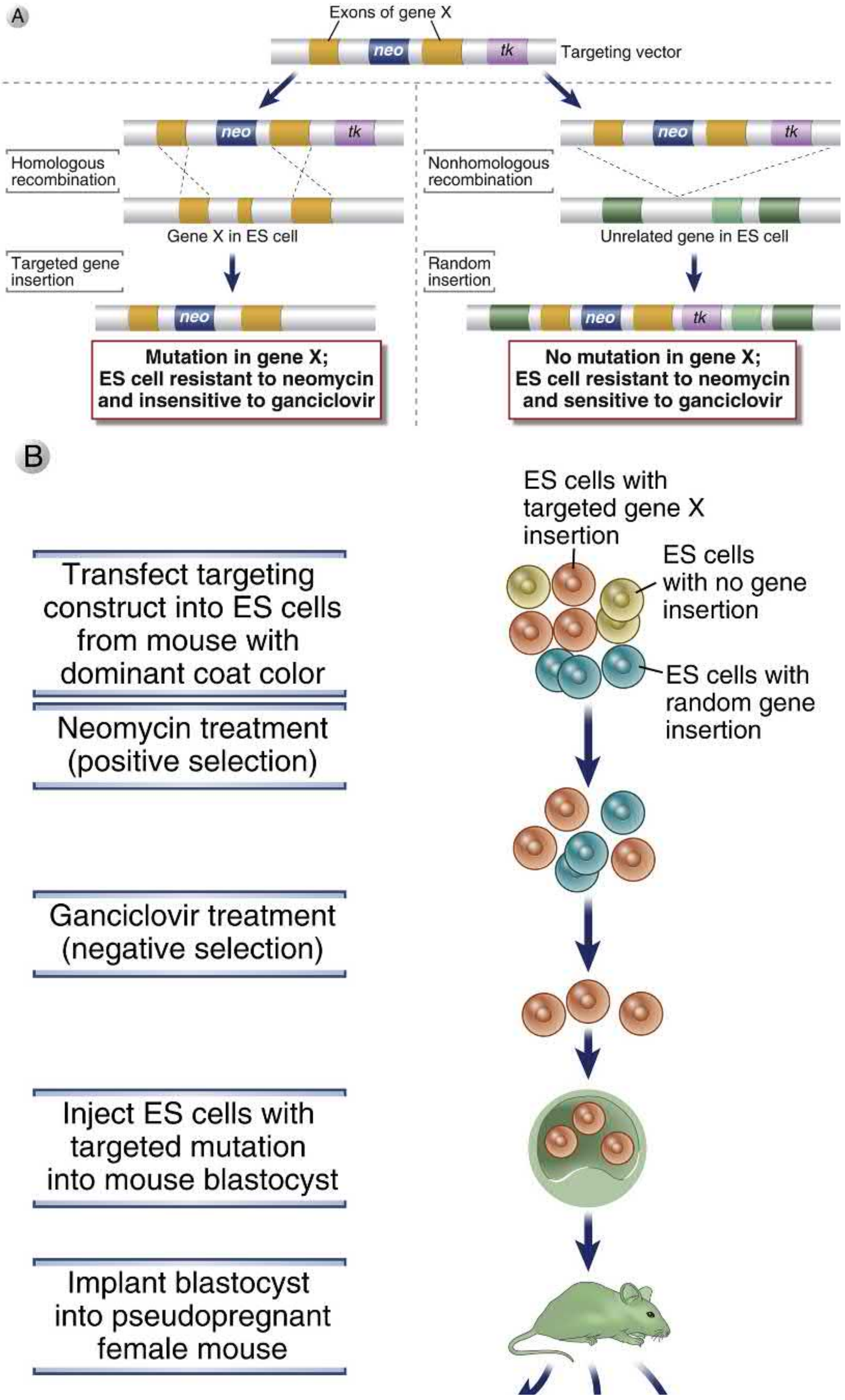
Although the conventional gene-targeting strategy has proved to be very useful in immunology research, the approach has some limitations. First, the mutation of one gene during development may be compensated for by altered expression of other gene products, and therefore the function of the targeted gene may be obscured. Second, in a conventional gene knockout mouse, the importance of a gene in only one tissue or at only one time during development cannot be easily assessed. Third, a functional selection marker gene, such as the *neo* gene, is permanently introduced into the animal genome, and this alteration may have unpredictable results on the phenotype of the animal. An important refinement of gene knockout technology that can overcome many of these drawbacks is a “conditional” targeting approach.

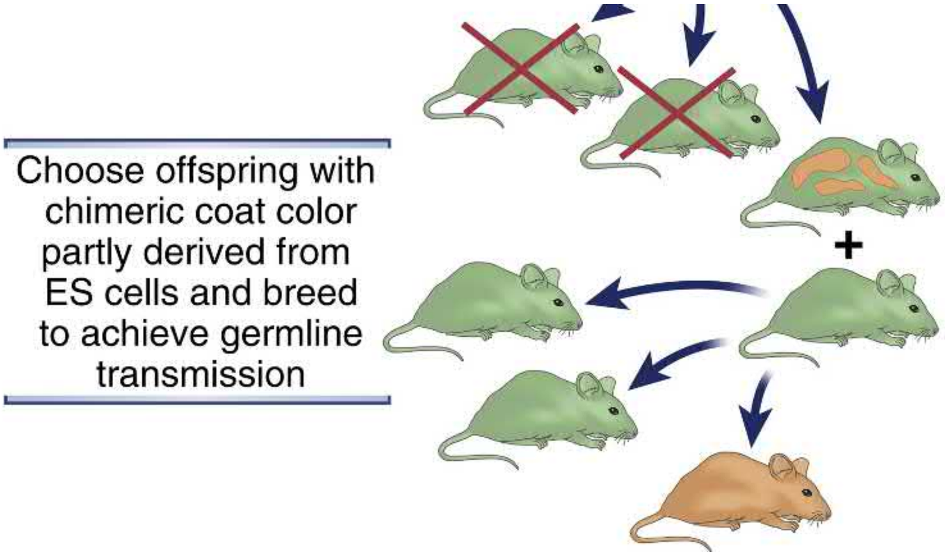
A commonly used conditional strategy takes advantage of the bacteriophage-derived Cre/*loxP* recombination system. The Cre enzyme is a DNA recombinase that recognizes a 34-bp sequence motif called *loxP,* and the enzyme mediates the deletion of gene segments flanked by two *loxP* sites in the same orientation. To generate mice with *loxP*-tagged genes, targeting vectors are constructed with one *loxP* site flanking the *neo* gene at one end and a second *loxP* site flanking the sequences homologous to the target at the other end. These vectors are transfected into ES cells, and mice carrying the *loxP*-flanked but still functional target gene are generated as described for conventional knockout mice. A second strain of mice carrying a *cre* transgene is then bred with the strain carrying the *loxP*-flanked (“floxed”) target gene. In the offspring, expression of Cre recombinase will mediate deletion of the target gene. Both the normal gene sequences and the *neo* gene will be deleted. Importantly, expression of the *cre* gene, and therefore deletion of the targeted gene, can be restricted to certain tissues or specified times by the use of *cre* transgene constructs with different promoters. For example, selective deletion of a gene only in macrophages and granulocytes can be accomplished by using a *cre* transgenic mouse in which *cre* is driven by a lysozyme promoter, or the selective loss of a gene only in regulatory T cells can be accomplished using a *foxp3* promoter driving a *cre* transgene. Alternatively, a steroid-inducible promoter can be used so that Cre expression and subsequent gene deletion occur only after mice are given a dose of dexamethasone. Many other variations on this technology have been devised to create conditional mutants. Cre/*loxP* technology also can be used to create knockin mice. In this case, *loxP* sites are placed in the targeting vector to flank the *neo* gene and the homologous sequences, but they do not flank the replacement (knockin) gene sequences. Therefore, after *cre*-

mediated deletion, the exogenous gene remains in the genome at the targeted site.

Gene knockin technology has been applied to create "reporter" mice in which cells that would normally express a particular protein will express a fluorescent molecule at the same time as the native protein. This is accomplished by replacing the native gene with a transgene that encodes the fluorescent reporter protein and the native protein, both under the control of the native promoter and enhancer. Reporter mice have been developed that allow the visualization of immune cells of particular subsets in vivo, such as mice in which cells producing a cytokine also express a fluorescent protein. These cells can be detected using intravital fluorescence microscopy. The cells expressing the reporter genes also can be isolated alive and subjected to functional studies ex vivo, even if the native gene reported is a nuclear transcription factor whose expression would otherwise be detectable only by methods that kill the cells. For example, live regulatory T cells can be isolated by FACS-sorting lymph nodes from a reporter mouse that expresses green fluorescent protein simultaneously with the transcription factor FOXP3.

A new approach to generating mutations in cell lines, as well as in ES cells, uses a modification of a bacterial defense system against foreign DNA called the **CRISPR** (Clustered Regularly Interspaced Short Palindromic Repeats) **Cas9** (CRISPR-associated nuclease 9) system. In the gene-editing variation of this, a guide RNA hybridizes with a chosen target DNA sequence and allows the Cas9 nuclease to generate a targeted double-stranded break. Although such a break can disrupt a gene, co-transfecting a plasmid with a mutated version of the target sequence allows efficient homologous recombination and the creation of a targeted knockin mutation. This is the most rapid approach available for the generation of knockout or knockin mutations in cell lines or in the germlines of experimental animals.


A
Exons of gene X
neo
tk
Targeting vector
Homologous recombination
Gene X in ES cell
Targeted gene insertion
Mutation in gene X;
ES cell resistant to neomycin
and insensitive to ganciclovir
Nonhomologous recombination
Unrelated gene in ES cell
Random insertion
No mutation in gene X;
ES cell resistant to neomycin
and sensitive to ganciclovir
B
Transfect targeting construct into ES cells from mouse with dominant coat color
Neomycin treatment (positive selection)
Ganciclovir treatment (negative selection)
Inject ES cells with targeted mutation into mouse blastocyst
Implant blastocyst into pseudopregnant female mouse
ES cells with targeted gene X insertion
ES cells with no gene insertion
ES cells with random gene insertion



Choose offspring with chimeric coat color partly derived from ES cells and breed to achieve germline transmission

FIG. A.5 Generation of gene knockout. **A,** The disruption of gene X in an embryonic stem *(ES)* cell is accomplished by homologous recombination. A population of ES cells is transfected with a targeting vector that contains sequences homologous to two exons of gene X flanking a neomycin resistance *(neo)* gene. The *neo* gene replaces or disrupts one of the exons of gene X on homologous recombination. The thymidine kinase *(tk)* gene in the vector will be inserted into the genome only if random, nonhomologous recombination occurs. **B,** The ES cells that were transfected by the targeting vector are selected by neomycin and ganciclovir so that only those cells with targeted insertion (homologous recombination) survive. These cells are then injected into a blastocyst, which is then implanted into the uterus of a pseudopregnant mouse. A chimeric mouse will develop in which some of the tissues are derived from the ES cell carrying the targeted mutation in gene X. These chimeric mice are identified by a mixed-color coat, including the color of the mouse strain from which the ES cells were derived and the color of the

mouse strain from which the blastocyst was derived. If the mutation is present in germ cells, it can be propagated by further breeding.

Methods for Studying T Lymphocyte Responses

Our current knowledge of the cellular events in T cell activation is based on a variety of experimental techniques in which different populations of T cells are activated by defined stimuli, and functional responses are measured. In vitro experiments have provided a great deal of information on the changes that occur in a T cell when it is stimulated by antigen. More recently, several techniques have been developed to study T cell proliferation, cytokine expression, and anatomic redistribution in response to antigen activation in vivo. The new experimental approaches have been particularly useful for the study of naive T cell activation and the localization of antigen-specific memory T cells after an immune response has waned. These in vivo approaches have largely been used in mice.

Polyclonal Activation of T Cells

Polyclonal activators of T cells bind to many or all T cell receptor (TCR) complexes regardless of specificity and activate the T cells in ways similar to peptide–major histocompatibility complex (MHC) complexes on antigen-presenting cells (APCs). Polyclonal activators are mostly used in vitro to activate T cells isolated from human blood or the lymphoid tissues of experimental animals. Polyclonal activators also can be used to activate T cells with unknown antigen specificities, and they can evoke a detectable response from mixed populations of naive T cells, even though the frequency of cells specific for any one antigen would be too low to elicit a detectable response. The polymeric carbohydrate-binding plant lectins, such as concanavalin-A and phytohemagglutinin (PHA), are a commonly used group of polyclonal T cell activators. These lectins bind specifically to certain sugar residues on T cell surface glycoproteins, including the TCR and CD3 proteins, and thereby stimulate the T cells. Antibodies specific for TCR-associated CD3 proteins also function as polyclonal activators of T cells and are now used more commonly than plant lectins. Often, these antibodies need to be immobilized on solid surfaces or beads or cross-linked with secondary anti-antibodies to induce optimal activation responses. Because soluble polyclonal activators do not provide costimulatory signals that are normally provided by APCs, they are often used together with stimulatory antibodies to receptors for costimulators, such as anti-CD28. Superantigens, another kind of polyclonal stimulus, bind to and activate all T cells that express particular types of TCR β chain (see Chapter 16, Fig. 16.3). T cells of any antigen specificity also can be stimulated with pharmacologic reagents, such as the combination of the phorbol ester PMA and the calcium ionophore ionomycin, that mimic signals generated by the TCR complex.

Antigen-Induced Activation of Polyclonal T Cell Populations

Polyclonal populations of normal T cells that are enriched for T cells specific for a particular antigen can be derived from the blood and peripheral lymphoid organs of individuals after immunization with the antigen. The immunization serves to expand the number of antigen-specific T cells, which can then be restimulated in vitro by adding antigen and MHC-matched APCs to the T cells. This approach can be used to study antigen-induced activation of a mixed population of previously activated (“primed”) T cells expressing many different TCRs, but naive T cells usually do not respond well to stimulation with antigen in vitro, probably because the frequency of cells specific for any antigen is very low.

Antigen-Induced Activation of T Cell Populations With a Single Antigen Specificity

Monoclonal populations of T cells, which express identical TCRs, have been useful for functional, biochemical, and molecular analyses. The limitation of these monoclonal populations is that they are maintained as long-term tissue culture lines and therefore may have phenotypically diverged from normal T cells in vivo. One type of monoclonal T cell population that has been used in experimental immunology is an antigen-specific T cell clone. Both helper and cytotoxic T lymphocyte clones have been established from mice and humans. Other monoclonal T cell populations used in the study of T cell activation include antigen-specific T cell hybridomas, which are produced like B cell hybridomas (see Fig. 5.9, Chapter 5), and tumor lines derived from T cells have been established in vitro after removal of malignant T cells from animals or humans with T cell leukemias or lymphomas. Although some tumor-derived lines express functional TCR complexes, their antigen specificities are not known and the cells are usually stimulated with polyclonal activators for experimental purposes. The Jurkat line, derived from a human T cell leukemia cell, is an example of a tumor line that is widely used as a model to study T cell signal transduction.

TCR transgenic mice are a source of homogeneous, phenotypically normal T cells with identical antigen specificities that are widely used for experimental studies. If the rearranged α and β chain genes of a single TCR of known specificity are expressed as a transgene in mice, a majority of the mature T cells in the mice will express that TCR. If the TCR transgene is crossed onto a RAG1- or RAG2-deficient background, no endogenous TCR gene expression occurs, and 100% of the T cells will express only the transgenic TCR. TCR transgenic T cells can be activated in vitro or in vivo with a single peptide antigen, and they can be identified by antibodies specific for the transgenic TCR. One of the unique advantages of TCR transgenic mice is that they permit the isolation of sufficient numbers of naive T cells of defined specificity to allow one to study functional responses to the first exposure to antigen. This advantage has allowed investigators to study the in vitro conditions under which antigen activation of naive T cells leads to differentiation into functional subsets such as Th1 and Th2 cells (see Chapter 9). Naive T cells from TCR transgenic mice also can be injected into normal syngeneic recipient mice, where they home to lymphoid tissues. The recipient mouse is then exposed to the antigen for which the transgenic TCR is specific. By use of antibodies that label the TCR transgenic T cells, it is possible to follow their expansion and differentiation in vivo and to isolate them for analysis of recall (secondary) responses to antigen ex vivo.

Methods to Enumerate and Study Functional Responses of T Cells

In the past, measurement of proliferation of T lymphocytes, like those of other cells, in vitro were often conducted by determining the amount of ^{3}H-labeled thymidine incorporated into the replicating DNA of cultured cells. Thymidine incorporation provides a quantitative measure of the rate of DNA synthesis, which is usually directly proportional to the rate of cell division. As use of radioactive isotopes in laboratory research has declined for safety and environmental reasons, other cellular proliferation assays have become more common and these can be used in vitro and in vivo.

Lymphocyte proliferation in vivo can be measured by injecting the thymidine analogue bromodeoxyuridine (BrdU) into animals, or having animals drink BrDU-containing water, and then staining cells harvested from the animals with anti-BrdU antibody to identify and enumerate nuclei that have incorporated BrdU into their DNA during DNA replication.

Fluorescent dyes can be used to study proliferation of T cells in vitro or in vivo. T cells are first labeled with chemically reactive lipophilic fluorescent esters and then the cell can be cultured in vitro under various experimental conditions or adoptively transferred into experimental animals. The dyes enter cells, form covalent bonds with cytoplasmic proteins, and then cannot leave the cells. One commonly used dye of this type is 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE), which can be detected in cells by standard flow cytometric techniques. Every time a cell divides, its dye content is halved, and therefore it is possible to determine whether the cultured T cells or the adoptively transferred T cells recovered from the lymphoid tissues of the recipient mouse have divided. It is also possible to estimate the number of doublings each T cell has gone through.

Peptide-MHC tetramers are used to enumerate T cells with a single antigen specificity isolated from blood or lymphoid tissues of experimental animals or humans. These tetramers contain four of the peptide-MHC complexes that the T cell would normally recognize on the surface of APCs. The tetramer is made by producing a soluble (non–membrane bound) class I MHC molecule to which is attached a small molecule called biotin by use of recombinant DNA technology. Biotin binds with high affinity to a protein called avidin, and each avidin molecule binds four biotin molecules. Thus, avidin forms a substrate for assembly of four biotin-conjugated MHC proteins. The MHC molecules can be loaded with a peptide of interest and thus stabilized, and the avidin molecule is labeled with a fluorochrome, such as FITC. This tetramer binds to T cells specific for the peptide-MHC complex with high enough avidity to label the T cells, even in suspension. This method is the only feasible approach for identification of antigen-specific T cells in humans. For instance, it is possible to identify and enumerate circulating HLA-A2–restricted T cells specific for an HIV peptide by staining blood cells with a tetramer of HLA-A2 molecules loaded with the peptide. The same technique is being used to enumerate and isolate T cells specific for self antigens in normal individuals and in patients with autoimmune diseases. Peptide-MHC tetramers that bind to a particular transgenic TCR also can be used to quantify the transgenic T cells in different tissues after adoptive transfer and antigen stimulation. The technique is now widely used with class I MHC molecules; in class I molecules, only one polypeptide is polymorphic, and stable molecules can be produced in vitro. This is more difficult for class II molecules because both chains are polymorphic and required for proper assembly, but class II–peptide tetramers have also been produced.

Cytokine secretion assays can be used to quantify cytokine-secreting effector T cells. The most commonly used methods are cytoplasmic staining of cytokines with analysis of the stained cells by flow cytometry and single-cell enzyme-linked immunosorbent assays (ELISpots). In these types of studies, antigen-induced activation and differentiation of T cells take place in vivo, and then T cells are isolated, restimulated with antigen or polyclonal activators, and tested for cytokine expression in vitro. Cytoplasmic staining of cytokines requires permeabilizing the cells so that fluorochrome-labeled antibodies specific for a particular cytokine can gain entry into the cell, and the stained cells are analyzed by flow cytometry. Cytokine expression by T cells specific for a particular antigen can be determined by additionally staining T cells with peptide-MHC tetramers or, in the case of TCR transgenic T cells, antibodies specific for the transgenic TCR. By use of a combination of CFSE and anti-cytokine antibodies, it is possible to examine the relationship between cell division and cytokine expression. In the ELISpot assay, T cells freshly isolated from blood or lymphoid tissues are cultured in plastic wells coated with antibody specific for a particular cytokine. As cytokines are secreted from individual T cells, they bind to the antibodies in discrete spots corresponding to the location of individual T cells. The spots are visualized by adding secondary enzyme-linked anti-Ig, as in a standard ELISA (see earlier), and the number of spots is counted to determine the number of cytokine-secreting T cells.

T Cell Receptor Repertoire Analyses

A powerful way to determine whether T cell clonal expansion has occurred or to determine clonal relatedness across different T cell populations is to interrogate the sequence of one or both chains of the TCR in T cell populations. Two broad approaches are used. On populations of T cells, next-generation sequencing of either genomic DNA or expressed RNA can be used to obtain the CDR3 sequences of the TCR α or β chain or of both chains. The most prominent clones, the "identifying" CDR3 sequence for a clone, and the V and J gene usages of individual clones can thus be determined, and this can help identify both clonal expansion and clonal relatedness between different T cell subpopulations in a given patient or mouse. Because only a very small number of research laboratories use TCR (in recombinant soluble form) for antigen discovery, in general identifying the matched TCR α and β chain at a single cell level is not commonly undertaken. However, the advent of new droplet capture techniques for the isolation of single cells has allowed the development of procedures for fairly easily sequencing TCR α and β chain genes from individual T cells. In single-cell approaches, knowledge of the specific TCR in each cell can also be correlated with the transcriptional profile of the given cell. In this manner, clonal expansion of different subsets of T cells in a disease setting can be assessed.

Methods for Studying B Lymphocyte Responses

Activation of Polyclonal B Cell Populations

A common approach to broadly interrogating B cell function in a non–antigen-specific manner is the use of anti-Ig antibodies as analogues of antigens, with the assumption that anti-Ig will bind to constant (C) regions of membrane Ig molecules on all B cells and will have the same biologic effects as an antigen that binds to the hypervariable regions of membrane Ig molecules on only the antigen-specific B cells. Thus, anti-Ig antibody is frequently used as a polyclonal activator of B lymphocytes, similar to the use of anti-CD3 antibodies as polyclonal activators of T lymphocytes, discussed earlier.

Antigen-Induced Activation of B Cell Populations With a Single Antigen Specificity

Antigen-specific B cells can be purified using magnetic beads coated with antigen, and antigen-specific proliferation assays can be performed on the purified cells; these assays have been used in patients with infectious diseases and in vaccination studies. While cloning and expansion methods for single B cells have been developed, they usually involve the use of CD40L-expressing monolayers, which results in the activation and differentiation of B cells and therefore limits the usefulness of the method. However, transgenic mice have been developed in which virtually all B cells express a transgenic Ig of known specificity, so that most of the B cells in these mice respond to the same antigen. Another approach has been to generate antigen receptor knockin mice, in which rearranged Ig H and L chain genes encoding a particular Ig are introduced into their endogenous loci. Such knockin animals have proved particularly useful for studying receptor editing.

Assays to Measure B Cell Proliferation and Antibody Production

Much of our knowledge of B cell activation is based on in vitro experiments, in which different stimuli are used to activate B cells and their proliferation and differentiation can be measured accurately. The same assays may be done with B cells recovered from mice exposed to different antigens or with homogeneous B cells expressing transgene-encoded antigen receptors.

B cell proliferation is measured by use of CFSE labeling or ^{3}H-labeled thymidine incorporation in vitro and BrdU labeling in vivo, as described earlier for T cell proliferation.

Antibody production is measured in two different ways: with assays for cumulative Ig secretion, which measure the amount of Ig that accumulates in the supernatant of cultured lymphocytes or in the serum of an immunized individual; and with single-cell assays, which determine the number of cells in an immune population that secrete Ig of a particular specificity or isotype. The most accurate, quantitative, and widely used technique to measure the total amount of Ig in a culture supernatant or serum sample is ELISA. By use of antigens bound to solid supports, it is possible to use ELISA to quantify the amount of antibody in a sample specific for a particular antigen. In addition, the availability of anti-Ig antibodies that detect Igs of different heavy or light chain classes allows measurement of the quantities of different isotypes in a sample. Other techniques to measure antibody levels include hemagglutination for anti-erythrocyte antibodies and complement-dependent lysis for antibodies specific for known cell types. Both assays are based on the demonstration that if the amount of antigen (i.e., cells) is constant, the concentration of antibody determines the amount of antibody bound to cells, and this is reflected in the degree of cell agglutination or subsequent binding of complement and cell lysis. Results from these assays are usually expressed as antibody titers, which are the dilution of the sample giving half-maximal effects or the dilution at which the endpoint of the assay is reached.

The ELISpot assay is used to enumerate cells producing antibody of a particular specificity. In this method, antigen is bound to the bottom of a well, antibody-secreting cells are added, and antibodies that have been secreted and are bound to the antigen are detected by an enzyme-linked anti-Ig antibody, as in an ELISA, in a semisolid medium. Each spot represents the location of an antibody-secreting cell. Single-cell assays provide a measure of the numbers of Ig-secreting cells, but they cannot accurately quantify the amount of Ig secreted by each cell or by the total population. The ELISA and ELISpot techniques can be adapted to assess affinity of antibodies, by the use of antigens with differing numbers of hapten moieties. In this way, affinity maturation can be assessed by testing serum or B cells sampled at different times during an immune response.

B Cell Receptor Repertoire Analyses

Interrogation of the sequence of Ig H and L chain genes in sorted B cell populations is a powerful way to determine whether B cell clonal expansion has occurred or to determine clonal relatedness across different B cell populations. Next-generation sequencing of either genomic DNA or of expressed RNA isolated from populations of B cells can be used to obtain the CDR3 sequences of the Ig H and L chains. The CDR3 sequences can then be used to identify the most prominent clones and the "identifying" CDR3 sequence and V and J gene usages of individual clones. This information can help identify both clonal expansion and clonal relatedness between different B cell populations in a given individual. By cloning single B cells and sequencing Ig H and L chain genes from single cells, it has been possible to create recombinant monoclonal antibodies from single B cells in humans and mice. These monoclonal antibodies have been used in determining autoreactivity, developing monoclonal antibody reagents against antigens from infectious agents and to purify unknown antigens that drive large B cell responses in human patients. Whereas previous single cell approaches were relatively labor intensive, the advent of new droplet capture techniques for the isolation of single cells has allowed the development of far more efficient procedures for sequencing Ig H and L chain genes from individual B cells.

Clinical Diagnostic Applications of Immunologic Assays

Many of the techniques discussed previously are used in clinical laboratories to determine the status of the immune system of patients. Here we will summarize some of the most common laboratory approaches for diagnosis of immunologic abnormalities. In many cases, abnormalities found by these approaches are followed with highly specialized tests, including molecular genetic analyses.

Flow Cytometry to Determine Numbers of Subsets of Circulating Immune Cells

Flow cytometry is routinely used to determine total numbers of B cells, T cells, natural killer (NK) cells, and T cells subsets (CD4$^+$ and CD8$^+$) in circulation (see Chapter 2, Fig. 2.7). Follow-up approaches include analyzing populations of naive and memory T cell subsets (CD45RA$^+$/RO$^+$), $\gamma\delta$ T cells, isotype switched memory B cells (CD27$^+$ IgM$^-$ IgD$^-$), and even subsets of T helper cells (Th1, Th2, Th17, Treg) depending on the context.

Assays for Innate Immunity

The **neutrophil oxidative burst assay** is commonly performed using a dihydrorhodamine (DHR) flow cytometric analysis and can be used to detect both overt chronic granulomatous disease as well as X-linked carriers of the disease In this test, DHR, which is a nonfluorescent lipid soluble compound, is added to suspensions of neutrophils, which are then stimulated by PMA. DHR can be oxidized by hydrogen peroxide produced during neutrophil respiratory burst by phagocyte oxidase or myeloperoxidase. DHR oxidation converts DHR into a green fluorescent cationic rhodamine compound, which localizes in mitochondria and can be detected by flow cytometry.

NK cell cytotoxicity assays evaluate ex vivo NK cell killing of a target cell population (e.g., cells lacking MHC). A low value suggests NK cell dysfunction and is useful in the evaluation of patients with recurrent infections (primarily viral), as well as in patients with suspected primary cause of hemophagocytic lymphohistiocytosis (HLH).

Assays for Humoral Immunity

Serologic detection of specific antibodies has been used for infectious disease diagnosis and blood grouping for well over a century. In infections, specific IgM antibodies indicate recent exposure to a pathogen, and IgG antibodies can be detected within 2 weeks of disease onset and persist decades later as well.

Serum protein electrophoresis can reveal decreased gamma globulins in immunodeficiency and monoclonal Ig peaks associated with malignant and premalignant clonal expansions of plasma cells.

Serum levels of different antibody classes, including IgG, IgA, IgM, and IgE as well as IgG subclasses, are usually determined by automated nephelometry, which involves mixing a dilution of patient's serum with antibodies specific for different Ig heavy chain classes, forming small immune complexes that are detected and quantified by measuring light scattering. Evaluation of the levels of IgG subclasses in serum is most helpful in patients who have normal to borderline low total IgG (≤400 mg/dL in the adult population).

Complement levels and function are quantified in several clinical contexts, including recurrent infections, recurrent angioedema, and/or autoimmune disease. In the setting of recurrent infections (particularly with encapsulated organisms such as *Neisseria*), a CH50 level is recommended as the initial screening test followed by more detailed pathway analysis in the setting of a low/absent CH50. The CH50 is a screening test for deficiency of classical or terminal pathways, and is determined by measuring the ability of a patent's serum to cause hemolysis of sheep erythrocytes pre-coated with complement-fixing antibodies. The dilution of the serum that results in 50% hemolysis is of the erythrocytes is the CH50.

Analysis of individual complement proteins is performed by nephelometry or variations of ELISAs. In the context of recurrent angioedema, a C4 level is often recommended as the initial screening test, followed by a C1 inhibitor level and function in the setting of a low C4 and/or a high level of clinical suspicion of an underlying C1 inhibitor deficiency. In the context of autoimmune disease, a low C3 and/or C4 level can be useful measures of ongoing immune complex formation.

Autoantibody screening for a range of specificities may be performed depending on the clinical context, using various techniques in which a patient's serum is tested for the presence of Ig that binds to purified antigens or to cells.

Vaccine responses are routinely measured to assess humoral immune function. Responses are determined by measuring serum levels of IgG specific to both T cell–dependent antigens (proteins or glycoproteins, e.g., vaccination against tetanus toxoid, diphtheria toxoid, and *Haemophilus influenzae* type B) and T cell–independent antigens (polysaccharides, e.g., Pneumovax). Titers are most commonly measured approximately 6 weeks after vaccination, and low titers may warrant further evaluation for an underlying B cell immunodeficiency.

Assays for Cellular Immunity

T cell receptor excision circles (TRECs), which are formed by V-D-J recombination during T cell maturation, are measured in a newborn blood screening assay that is now mandatory in most states in the United States. TREC levels are used to assess recent T cell output from the thymus, and a low level indicates deficiency of T cells, which warrants further evaluation for severe combined immunodeficiency (SCID).

T cell proliferation assays are performed to assess T cell function and evaluated by stimulating cells ex vivo with mitogens such as pokeweed mitogen (PWM) and PHA, specific antigens (*Candida* and tetanus toxoid are commonly used), or antibodies to CD3 and CD28. Robust cellular proliferation to these stimuli suggests intact T cell function.

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